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(54) Title: CDR-GRAFTED ANTI-TISSUE FACTOR ANTIBODIES AND METHODS OF USE THEREOF

(57) Abstract

The present invention provides CDR-grafted antibodies against human tissue factor that retain the high binding affinity of rodent monoclonal antibodies against tissue factor but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.

CDR-GRAFTED ANTI-TISSUE FACTOR

1   ANTIBODIES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

5   Monoclonal antibodies capable of inhibiting
tissue factor (TF) are useful as anticoagulants.
Conventional rodent monoclonal antibodies, however, have
limited use in human therapeutic and diagnostic
applications due to immunogenicity and short serum half-

10   life. The present invention provides CDR-grafted
monoclonal antibodies against TF that retain the high
binding affinity of rodent antibodies but have reduced
immunogenicity. The present humanized antibodies are
potent anticoagulants and are thus useful in the

15   treatment and prophylaxis of human thrombotic disease.
The invention also provides methods of making the CDR-
grafted antibodies and pharmaceutical compositions for
the attenuation or prevention of coagulation.

BACKGROUND OF THE INVENTION

20

              The coagulation of blood involves a cascading
series of reactions leading to the formation of fibrin.
The coagulation cascade consists of two overlapping

25   pathways, both of which are required for hemostasis.
The intrinsic pathway comprises protein factors present
in circulating blood, while the extrinsic pathway
requires tissue factor, which is expressed on the cell
surface of a variety of tissues in response to vascular

30   injury. Davie et al., 1991, Biochemistry 30:10363.
Agents that interfere with the coagulation cascade, such

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as heparin and coumarin derivatives, have well-known
1 therapeutic uses in the prophylaxis of venous
   thrombosis. Goodman and Gilman, eds., 1980, The
   Pharmacological Basis of Therapeutics, MacMillan
   Publishing Co., Inc., New York.
5   Tissue factor (TF) has been investigated as a
   target for anticoagulant therapy. TF is a membrane
   glycoprotein that functions as a receptor for factor VII
   and VIIa and thereby initiates the extrinsic pathway of
   the coagulation cascade in response to vascular injury.
10 In addition to its role in the maintenance of hemostasis
   by initiation of blood clotting, TF has been implicated
   in pathogenic conditions. Specifically, the synthesis
   and cell surface expression of TF has been implicated in
   vascular disease (Wilcox et al., 1989, Proc. Natl. Acad.
15 Sci. 86:2839) and gram-negative septic shock (Warr et
   al., 1990, Blood 75:1481).
           Ruf et al. (1991, Thrombosis and Haemostasis
   66:529) characterized the anticoagulant potential of
   murine monoclonal antibodies against human TF. The
20 inhibition of TF function by most of the monoclonal
   antibodies that were assessed was dependent upon the
   dissociation of the TF/VIIa complex that is rapidly
   formed when TF contacts plasma. Such antibodies were
   thus relatively slow inhibitors of TF in plasma. One
25 monoclonal antibody, TF8-5G9, was capable of inhibiting
   the TF/VIIa complex without dissociation of the complex,
   thus providing an immediate anticoagulant effect in
   plasma. Ruf et al. suggest that mechanisms that
   inactivate the TF/VIIa complex, rather than prevent its
30 formation, may provide strategies for interruption of
   coagulation in vivo.

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The therapeutic use of monoclonal antibodies
1 against TF is limited in that currently available
monoclonals are of rodent origin. The use of rodent
antibodies in human therapy presents numerous problems,
the most significant of which is immunogenicity.
5 Repeated doses of rodent monoclonal antibodies have been
found to elicit an anti-immunoglobulin response termed
human anti-mouse antibody (HAMA), which can result in
immune complex disease and/or neutralization of the
therapeutic antibody. See, e.g., Jaffers et al. (1986)
10 Transplantation 41:572. While the use of human
monoclonal antibodies would address this limitation, it
has proven difficult to generate large amounts of human
monoclonal antibodies by conventional hybridoma
technology.
15   Recombinant technology has been used in an
effort to construct "humanized" antibodies that maintain
the high binding affinity of rodent monoclonal
antibodies but exhibit reduced immunogenicity in humans.
Chimeric antibodies have been produced in which the
20 variable (V) region of a mouse antibody is combined with
the constant (C) region of a human antibody in an effort
to maintain the specificity and affinity of the rodent
antibody but reduce the amount of protein that is non-
human and thus immunogenic. While the immune response
25 to chimeric antibodies is generally reduced relative to
the corresponding rodent antibody, the immune response
cannot be completely eliminated, because the mouse V
region is capable of eliciting an immune response.
Lobuglio et al. (1989) Proc. Natl. Acad. Sci. 86:4220;
30 Jaffers et al. (1986) Transplantation 41:572.

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In a recent approach to reducing
1 immunogenicity of rodent antibodies, only the rodent
complementarity determining regions (CDRs), rather than
the entire V domain, are transplanted to a human
antibody. Such humanized antibodies are known as CDR-
5 grafted antibodies. CDRs are regions of
hypervariability in the V regions that are flanked by
relatively conserved regions known as framework (FR)
regions. Each V domain contains three CDRs flanked by
four FRs. The CDRs fold to form the antigen binding
10 site of the antibody, while the FRs support the
structural conformations of the V domains. Thus by
transplanting the rodent CDRs to a human antibody, the
antigen binding domain can theoretically also be
transferred. Owens et al. (1994) J. Immunol. Methods
15 168:149 and Winter et al. (1993) Immunology Today 14:243
review the development of CDR-grafted antibodies.

Orlandi et al. (1989) Proc. Natl. Acad. Sci.
USA 86:3833 constructed a humanized antibody against the
relatively simple hapten nitrophenacetyl (NP). The CDR-
20 grafted antibody contained mouse CDRs and human FRs, and
exhibited NP binding activity similar to the native
mouse antibody. However, the construction of CDR-
grafted antibodies recognizing more complex antigens has
resulted in antibodies having binding activity
25 significantly lower than the native rodent antibodies.
In numerous cases it has been demonstrated that the mere
introduction of rodent CDRs into a human antibody
background is insufficient to maintain full binding
activity, perhaps due to distortion of the CDR
30 conformation by the human FR.

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For example, Gorman et al. (1991) Proc. Natl.
1   Acad. Sci. 88:4181 compared two humanized antibodies
against human CD4 and observed considerably different
avidies depending upon the particular human framework
region of the humanized antibody. Co et al. (1991)
5   Proc. Natl. Acad. Sci. USA 88:2869 required a refined
computer model of the murine antibody of interest in
order to identify critical amino acids to be considered
in the design of a humanized antibody. Kettleborough et
al. (1991) Protein Engineering 4:773 report the
10 influence of particular FR residues of a CDR-grafted
antibody on antigen binding, and propose that the
residues may directly interact with antigen, or may
alter the conformation of the CDR loops. Similarly,
Singer et al. (1993) J. Immunol. 150:2844 report that
15 optimal humanization of an anti-CD18 murine monoclonal
antibody is dependent upon the ability of the selected
FR to support the CDR in the appropriate antigen binding
conformation. Accordingly, recreation of the antigen-
binding site requires consideration of the potential
20 intrachain interactions between the FR and CDR, and
manipulation of amino acid residues of the FR that
maintain contacts with the loops formed by the CDRs.
While general theoretical guidelines have been proposed
for the design of humanized antibodies (see, e.g., Owens
25 et al.), in all cases the procedure must be tailored and
optimized for the particular rodent antibody of
interest.

There is a need in the art for humanized
antibodies with reduced immunogenicity and comparable
30 binding affinity relative to the parent rodent antibody
for various therapeutic applications. In particular,

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there is a need for a humanized antibody against human
1 tissue factor having anticoagulant activity and useful
in the treatment and prevention of thrombotic disease.

SUMMARY OF THE INVENTION
5

The present invention is directed to CDR-
grafted antibodies capable of inhibiting human tissue
factor wherein the CDRs are derived from a non-human
monoclonal antibody against tissue factor and the FR and
10 constant (C) regions are derived from one or more human
antibodies. In a preferred embodiment, the murine
monoclonal antibody is TF8-5G9.
In another embodiment, the present invention
provides a method of producing a CDR-grafted antibody
15 capable of inhibiting human tissue factor which method
comprises constructing one or more expression vectors
containing nucleic acids encoding CDR-grafted antibody
heavy and light chains, transfecting suitable host cells
with the expression vector or vectors, culturing the
20 transfected host cells, and recovering the CDR-grafted
antibody.
The present invention also provides a method
of attenuation of coagulation comprising administering a
CDR-grafted antibody capable of inhibiting human tissue
25 factor to a patient in need of such attenuation.
The present invention further provides a
method of treatment or prevention of thrombotic disease
comprising administering a CDR-grafted antibody capable
of inhibiting human tissue factor to a patient in need
30 of such treatment or prevention. In a preferred

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embodiment, the thrombotic disease is intravascular

1   coagulation, arterial restenosis or arteriosclerosis.
        Another embodiment of the present invention is
    directed to a pharmaceutical composition comprising CDR-
    grafted antibodies capable of inhibiting human tissue

5   factor and further comprising a pharmaceutically
    acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

10   Fig. 1 provides the nucleotide and deduced
    amino acid sequences of the heavy chain of murine
    monoclonal antibody TF8-5G9.
            Fig. 2 provides the nucleotide and deduced
    amino acid sequences of the light chain of murine

15 monoclonal antibody TF8-5G9.
            Fig. 3 is a graph depicting the ability of
    CDR-grafted antibody TF8HCDR1 x TF8LCDR1 to bind to
    human tissue factor and to compete with murine
    monoclonal antibody TF85G9 for binding to tissue factor.

20 Solid symbols indicate direct binding of TF8HCDR1 x
    TF8LCDR1 and the positive control chimeric TF85G9 to
    tissue factor. Open symbols indicate competition
    binding of TF8HCDR1 x TF8LCDR1 or chimeric TF85G9 with
    murine monoclonal antibody TF85G9.

25   Fig. 4 presents the DNA sequence of expression
    vector pEe6TF8HCDR20 and the amino acid sequence of the
    coding regions of the CDR-grafted heavy chain TF8HCDR20.
            Fig. 5 presents the DNA sequence of expression
    vector pEe12TF8LCDR3 and the amino acid sequence of the

30 coding regions of the CDR-grafted light chain TF8LCDR3.

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Fig. 6 is a graph depicting the ability of
1 CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to bind to
human tissue factor.

Fig. 7 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to compete
5 with murine monoclonal antibody TF85G9 for binding to
tissue factor.

Fig. 8 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to inhibit
factor X activation.

10   Fig. 9 provides expression vector
pEe6TF8HCDR20 resulting from the subcloning of CDR-
grafted heavy chain TF8HCDR20 into myeloma expression
vector pEehCMV-BglI. The following abbreviations are
used: VH is the CDR-grafted heavy chain variable
.15 region; Cγ4 is the human IgG4 constant region; pA is the
polyadenylation signal; ampR is the ß-lactamase gene;
and hCMV is human cytomegalovirus.

Fig. 10 provides expression vector
pEe12TF8LCDR3 resulting from the subcloning of CDR-
20 grafted light chain TF8LCDR3 into myeloma expression
vector pEe12. The following abbreviations are used: VL
is the CDR-grafted light chain variable region; CK is
the human kappa constant region; SVE is the SV40 early
promoter; GS is glutamine synthetase cDNA. Other
25 abbreviations are as noted in Fig. 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides CDR-grafted
30 antibodies capable of inhibiting human tissue factor
wherein the CDRs are derived from a non-human monoclonal

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antibody against tissue factor and the FR and C regions
1   are derived from one or more human antibodies. The
     present invention further provides methods of making and
     using the subject CDR-grafted antibodies.

            In accordance with the present invention, the
5   CDR-grafted antibody is an antibody in which the CDRs
     are derived from a non-human antibody capable of binding
     to and inhibiting the function of human tissue factor,
     and the FR and C regions of the antibody are derived
     from one or more human antibodies. The CDRs derived
10   from the non-human antibody preferably have from about
     90% to about 100% identity with the CDRs of the non-
     human antibody, although any and all modifications,
     including substitutions, insertions and deletions, are
     contemplated so long as the CDR-grafted antibody
15   maintains the ability to bind to and inhibit tissue
     factor. The regions of the CDR-grafted antibodies that
     are derived from human antibodies need not have 100%
     identity with the human antibodies. In a preferred
     embodiment, as many of the human amino acid residues as
20   possible are retained in order than immunogenicity is
     negligible, but the human residues, in particular
     residues of the FR region, are substituted as required
     and as taught hereinbelow in accordance with the present
     invention. Such modifications as disclosed herein are
25   necessary to support the antigen binding site formed by
     the CDRs while simultaneously maximizing the
     humanization of the antibody.

            Non-human monoclonal antibodies against human
     tissue factor from which the CDRs can be derived are
30   known in the art (Ruf et al., 1991; Morrisey et al.,
     1988, Thrombosis Research 52:247) or can be produced by

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well-known methods of monoclonal antibody production
1 (see, e.g. Harlow et al., eds., 1988, Antibodies, A
Laboratory Manual, Cold Spring Harbor Laboratories, Cold
Spring Harbor, New York). Purified human tissue factor
against which monoclonal antibodies can be raised is
5 similarly well-known (Morrisey et al., 1987, Cell
50:129) and available to the skilled artisan. Murine
monoclonal antibodies, and in particular murine
monoclonal antibody TF8-5G9 disclosed by Ruf et al. and
Morrisey et al., 1988, Thrombosis Research 52:247, and
10 U.S. Patent No. 5,223,427 are particularly preferred.
        The ordinarily skilled artisan can determine
the sequences of the CDRs by reference to published
scientific literature or sequence databanks, or by
cloning and sequencing the heavy and light chains of the
15 antibodies by conventional methodology. In accordance
with the present invention, the cDNA and amino acid
sequences of the heavy chain (SEQ ID NOS:1 and 2,
respectively) and light chain (SEQ ID NOS:3 and 4,
respectively) of murine monoclonal antibody TF8-5G9 are
20 provided. The cDNA and deduced amino acid sequence of
the murine TF8-5G9 heavy chain is provided at Figure 1.
The cDNA and deduced amino acid sequence of the murine
TF8-5G9 light chain is provided at Figure 2.
        Each of the heavy and light chain variable
25 regions contain three CDRs that combine to form the
antigen binding site. The three CDRs are surrounded by
four FR regions that primarily function to support the
CDRs. The sequences of the CDRs within the sequences of
the variable regions of the heavy and light chains can
30 be identified by computer-assisted alignment according
to Kabat et al. (1987) in Sequences of Proteins of

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Immunological Interest, 4th ed., United States
1 Department of Health and Human Services, US Government
   Printing Office, Washington, D.C., or by molecular
   modeling of the variable regions, for example utilizing
   the ENCAD program as described by Levitt (1983) _J. Mol._
5 _Biol._ _168_:595.

         In a preferred embodiment the CDRs are derived
from murine monoclonal antibody TF8-5G9. The preferred
heavy chain CDRs have the following sequences:

10   CDR1   DDYMH   (SEQ ID NO:5)
            CDR2   LIDPENGNTIYDPKFQG (SEQ ID NO:6)
            CDR3   DNSYYFDY   (SEQ ID NO:7)

The preferred light chain CDRs have the following
15 sequences:

            CDR1   KASQDIRKYLN   (SEQ ID NO:8)
            CDR2   YATSLAD   (SEQ ID NO:9)
            CDR3   LQHGESPYT   (SEQ ID NO:10)
20
The sequences of the CDRs of the murine or other non-
human antibody, and in particular the sequences of the
CDRs of TF8-5G9, may be modified by insertions,
substitutions and deletions to the extent that the CDR-
25 grafted antibody maintains the ability to bind to and
inhibit human tissue factor. The ordinarily skilled
artisan can ascertain the maintenance of this activity
by performing the functional assays described
hereinbelow. The CDRs can have, for example, from about
30 50% to about 100% homology to the CDRs of SEQ ID NOS:5-
10. In a preferred embodiment the CDRs have from about

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80% to about 100% homology to the CDRs of SEQ ID NOS:5-
1 10.   In a more preferred embodiment the CDRs have from
about 90% to about 100% homology to the CDRs of SEQ ID
NOS:5-10.   In a most preferred embodiment the CDRs have
from about 100% homology to the CDRs of SEQ ID NOS:5-10.

5   The FR and C regions of the CDR-grafted
antibodies of the present invention are derived from one
or more human antibodies.   Human antibodies of the same
class and type as the antibody from which the CDRs are
derived are preferred.   The FR of the variable region of

10 the heavy chain is preferably derived from the human
antibody KOL (Schmidt et al., 1983, Hoppe-Seyler's Z.
Physiol. Chem. 364:713)   The FR of the variable region
of the light chain is preferably derived from the human
antibody REI (Epp et al., 1974, Eur. J. Biochem.

15 45:513).   In accordance with the present invention, it
has been discovered that certain residues of the human
FR are preferably replaced by the corresponding residue
of the non-human antibody from which the CDRs are
derived.   For example, certain FR residues of TF8-5G9

20 are preferably retained to achieve optimal binding to
antigen.

           For convenience, the numbering scheme of Kabat
et al. has been adopted herein.   Residues are designated
by lower case numbers or hyphens as necessary to conform

25 the present sequences to the standard Kabat numbered
sequence.

           In accordance with the present invention,
residues that are retained in the FR region, i.e
residues that are not replaced by human FR residues, are

30 determined according to the following guidelines.
Residues that are idiosyncratic to the parent antibody,

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e.g. TF8-5G9, relative to a human consensus sequence of
1   Kabat et al, are retained. Residues of the parent
    antibody that are in agreement with the consensus
    sequence are retained if the corresponding residue of
    the human antibody, e.g. KOL or REI, is idiosyncratic.
5   Residues that are part of the antibody loop canonical
    structures defined by Chothia et al. (1989) Nature
    342:877, such as residue 71 of the heavy and light
    chains, are retained. FR residues predicted to form
    loops, such as residues 28-30 of the heavy chain, are
10 retained. FR residues predicted to influence the
    conformation of the CDRs such as residues 48 and 49
    preceding CDR2 of the heavy chain, are retained.
    Residues that have been demonstrated to be critical in
    the humanization of other antibodies may also be
15 retained. The foregoing guidelines are followed to the
    extent necessary to support the antigen binding site
    formed by the CDRs while simultaneously maximizing the
    humanization of the antibody.

            The amino acid sequence of a representative
20 CDR-grafted heavy chain variable region derived from
    murine monoclonal antibody TF8-5G9 and human antibody
    KOL is shown below. The CDR-grafted heavy chain is
    designated TF8HCDR1; murine residues were retained in
    the FR at residues 6, 17, 23, 24, 28, 29, 30, 48, 49,
25 68, 71, 73, 78, 88 and 91. CDRs are underlined.
```
         10        20        30    35ab              50
QVQLVQSGGG VVQPGRLLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIG
52abc      60        70            80 82abc       90
LIDP--ENGNTIYD PKFQGRFSIS ADTSK--NTAFL QMDSLRPEDTAVY
100        110
```
30 YCARDNSYYF DYWGQGTPVT VSS (SEQ ID NO:11)

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The amino acid sequence of a representative
1 CDR-grafted light chain variable region derived from
   murine monoclonal antibody TF8-5G9 and human antibody
   REI is shown below. The CDR-grafted light chain is
   designated TF8LCDR1; murine residues were retained in
5 the FR at residues 39, 41, 46 and 105. CDRs are
   underlined.

          10   20   30   40   50
      DIQMTQSPSS LSASVGDRVT ITCKASQDIR KYLNWYQQK WKAPKTLIYY
10   60   70   80   90   100
      ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLQ HGESPYTFGQ

   GTKLEITR (SEQ ID NO:12)

       A CDR-grafted antibody containing variable
15 regions TF8HCDR1 and TF8LCDR1 has been demonstrated in
   accordance with the present invention to be as effective
   as murine monoclonal antibody TF8-5G9 in binding to
   human tissue factor. It has been further discovered in
   accordance with the present invention, by examination of
20 the molecular structure of murine monoclonal antibody
   TF8-5G9, and by design, construction, and analysis of
   CDR-grafted antibodies, that the FR regions can be
   further humanized without the loss of antigen binding
   activity. In particular, the FR region may retain the
25 human FR residue at residues 6, 17, 68, 73 and 78 of the
   heavy chain, and residues 39, 41, 16 and 105 of the
   light chain, with maintenance of antigen binding
   activity.
       In a most preferred embodiment, the heavy
30 chain variable region contains a FR derived from human
   antibody KOL in which murine monoclonal antibody TF8-5G9

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residues are retained at amino acids 23, 24, 28, 29, 30,
1 48, 49, 71, 88 and 91. The preferred heavy chain
   variable region is designated TF8HCDR20 and has the
   following sequence.

```
5          10          20          30    35ab            50
       QVQLVESGGG VVQPGRSLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIGL


       52abc       60          70          80 82abc      90          100
       IDP--ENGNTIYD PKFQGRFTIS ADNSKNTLFL QMDSLRPEDTAVY YCARDNSYYF


10          110
       DYWGQGTPVT VSS  (SEQ ID NO:13)
```

        In a most preferred embodiment, the light
   chain variable region contains a FR derived from human
15 antibody REI in which murine monoclonal antibody TF8-5G9
   residues are retained at amino acids 39 and 105. The
   preferred light chain variable region is designated
   TF8LCDR20 and has the following sequence.

```
           10          20          30          40          50
        DIQMTQSPSS LSASVGDRVT ITCKASQDIR KYLNWYQQKP GKAPKLLIYY
20          60          70          80          90          100
        ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLQ HGESPYTFGQ
        GTKLEITR  (SEQ ID NO:14)
```

        It is within the ken of the ordinarily skilled
25 artisan to make minor modifications of the foregoing
   sequences, including amino acid substitutions, deletions
   and insertions. Any such modifications are within the
   scope of the present invention so long as the resulting
   CDR-grafted antibody maintains the ability to bind to
30 and inhibit human tissue factor. The ordinarily skilled
   artisan can assess the activity of the CDR-grafted

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antibody with reference to the functional assays
1 described hereinbelow.

    The human constant region of the CDR-grafted
antibodies of the present invention is selected to
minimize effector function. The intended use of the
5 CDR-grafted antibodies of the present invention is to
block the coagulation cascade by inhibition of tissue
factor, and thus antibody effector functions such as
fixation of complement are not desirable. Antibodies
with minimal effector functions include IgG2, IgG4, IgA,
10 IgD and IgE. In a preferred embodiment of the present
invention, the heavy chain constant region is the human
IgG4 constant region, and the light chain constant
region is the human IgG4 kappa constant region.

    In that effector functions may not be
15 desirable for therapeutic uses, the present invention
further contemplates active fragments of the CDR-grafted
antibodies, and in particular Fab fragments and $F(ab')_2$
fragments. Active fragments are those fragments capable
of inhibiting human tissue factor. Fab fragments and
20 $F(ab')_2$ fragments may be obtained by conventional means,
for example by cleavage of the CDR-grafted antibodies of
the invention with an appropriate proteolytic enzyme
such as papain or pepsin, or by recombinant production.
The active fragments maintain the antigen binding sites
25 of the CDR-grafted antibodies and thus are similarly
useful therapeutically.

    The ability of the CDR-grafted antibodies
designed and constructed as taught in accordance with
the present invention to bind and inhibit human tissue
30 factor can be assessed by functional assays. For
example, in a rapid and convenient assay, expression

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vectors containing nucleic acids encoding the CDR-
grafted heavy and light chains can be co-transfected
into suitable host cells and transiently expressed. The
resulting antibodies can be assessed by standard assays
for ability to bind human tissue factor, and for ability
to compete for binding to tissue factor with the non-
human antibody from which the CDRs are derived.

For example, transient expression of nucleic
acids encoding the CDR-grafted heavy and light chains in
COS cells provides a rapid and convenient system to test
antibody gene expression and function. Nucleic acids
encoding the CDR-grafted heavy and light chains,
respectively, are cloned into a mammalian cell
expression vector, for example pSG5, described by Green
et al. (1988) Nucleic Acids Res. 16:369 and commercially
available from Stratagene Cloning Systems, La Jolla, CA.
The pSG5 expression vector provides unique restriction
sites for the insertion of the heavy and light chain
genes, and in vivo expression is under the control of
the SV40 early promoter. Transcriptional termination is
signaled by the SV40 polyadenylation signal sequence.

The pSG5-based expression vectors containing
nucleic acids encoding the heavy and light chains are
cotransfected into COS cells and cultured under
conditions suitable for transient expression. Cell
culture media is then harvested and examined for
antibody expression, for example by an enzyme linked
immunosorbent assay (ELISA), to determine that suitable
levels of antibody have been produced. An ELISA may
then be used to assess the ability of the CDR-grafted
antibody to bind to human tissue factor. Human tissue
factor is immobilized on a microtiter plate and the COS

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cell supernatant containing the CDR-grafted antibody is
1 added followed by an incubation at room temperature for
about one hour. The plates are then washed with a
suitable detergent-containing buffer such as phosphate
buffered saline (PBS)/Tween, followed by the addition of
5 the components of a suitable detection system. For
example, horseradish peroxidase conjugated goat anti-
human kappa chain polyclonal antibody is added, followed
by washing, followed by addition of substrate for
horseradish peroxidase, and detection. The CDR-grafted
10 antibodies within the scope of the present invention are
those which are capable of binding to human tissue
factor to a degree comparable to the non-human antibody
from which the CDRs are derived as determined by the
foregoing assay.

15   The ability of the CDR-grafted antibodies to
inhibit the activity of human tissue factor in vivo can
be conveniently assessed by the following in vitro assay
that mimics in vivo coagulation events. In response to
vascular injury in vivo, tissue factor binds to factor
20 VII and facilitates the conversion of factor VII to a
serine protease (factor VIIa). The factor VIIa-tissue
factor complex converts factor X to a serine protease
(factor Xa). Factor Xa forms a complex with factor Va
(from the intrinsic coagulation pathway), resulting in
25 the conversion of prothrombin to thrombin, which in turn
results in the conversion of fibrinogen to fibrin. In a
convenient in vitro functional assay, tissue factor is
incubated in the presence of factor VIIa and the CDR-
grafted anti-tissue factor antibody produced in the
30 transient expression system described above. Factor X
is added and the reaction mixture is incubated, followed

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by an assay for factor Xa activity utilizing a

1 chromogenic substrate for factor Xa (Spectrozyme FXa,
American Diagnostica, Inc., Greenwich, CT). The ability
of the CDR-grafted antibody to inhibit factor X
activation thus provides a measure of the ability of the

5 CDR-grafted antibody to inhibit the activity of human
tissue factor.

The CDR-grafted antibodies within the scope of
the present invention are those which are capable of
inhibiting human tissue factor to a degree comparable to

10 the non-human antibody from which the CDRs are derived
as determined by the foregoing assay. In one
embodiment, the CDR-grafted antibody has at least 50% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a preferred embodiment, the CDR-grafted

15 antibody has at least 70% of the inhibitory activity of
TF8-5G9 for human tissue factor. In a more preferred
embodiment, the CDR-grafted antibody has at least 80% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a most preferred embodiment, the CDR-grafted

20 antibody has at least 90% of the inhibitory activity of
TF8-5G9 for human tissue factor.

In another embodiment, the present invention
provides a method of producing a CDR-grafted antibody
capable of inhibiting human tissue factor. The method

25 comprises constructing an expression vector containing a
nucleic acid encoding the CDR-grafted antibody heavy
chain and an expression vector containing a nucleic acid
encoding the CDR-grafted antibody light chain,
transfecting suitable host cells with the expression

30 vectors, culturing the transfected host cells under
conditions suitable for the expression of the heavy and

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light chains, and recovering the CDR-grafted antibody.

1 Alternately, one expression vector containing nucleic acids encoding the heavy and light chains may be utilized.

Standard molecular biological techniques, for

5 example as disclosed by Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, Cold Spring Harbor, NY may be used to obtain nucleic acids encoding the heavy and light chains of the CDR-grafted antibodies of the present invention.

10 A nucleic acid encoding the CDR-grafted variable domain may be constructed by isolating cDNA encoding the antibody to be humanized, e.g. murine monoclonal antibody TF8-5G9, by conventional cloning methodology from the hybridoma producing the antibody, or by

15 polymerase chain reaction (PCR) amplification of the variable region genes, as described for example by Winter et al., followed by site-directed mutagenesis to substitute nucleotides encoding the desired human residues into the FR regions. Alternately, the cDNA

20 encoding the human antibody can be isolated, followed by site-directed mutagenesis to substitute nucleotides encoding the desired murine residues into the CDRs.

Nucleic acids encoding the CDR-grafted variable domain may also be synthesized by assembling

25 synthetic oligonucleotides, for example utilizing DNA polymerase and DNA ligase. The resulting synthetic variable regions may then be amplified by PCR. Nucleic acids encoding CDR-grafted variable domains may also be constructed by PCR strand overlap methods that are known

30 in the art and reviewed by Owens et al.

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      Accordingly, having determined the desired
1 amino acid sequences of the CDR-grafted variable domains
in accordance with the present invention, the ordinarily
skilled artisan can obtain nucleic acids encoding the
variable domains. Further, the skilled artisan is aware
5 that due to the degeneracy of the genetic code, various
nucleic acid sequences can be constructed that encode
the CDR-grafted variable domains. All such nucleic acid
sequence are contemplated by the present invention.

      The nucleic acids encoding the CDR-grafted
10 variable domains are linked to appropriate nucleic acids
encoding the human antibody heavy or light chain
constant region. Nucleic acid sequences encoding human
heavy and light chain constant regions are known in the
art. It is within the ken of the ordinarily skilled
15 artisan to include sequences that facilitate
transcription, translation and secretion, for example
start codons, leader sequences, the Kozak consensus
sequence (Kozak, 1987, _J. Mol. Biol._ **196**:947) and the
like, as well as restriction endonuclease sites to
20 facilitate cloning into expression vectors.

      The present invention thus further provides
nucleic acids encoding the heavy and light chains of
CDR-grafted antibodies capable of inhibiting human
tissue factor wherein the CDRs are derived from a murine
25 monoclonal antibody against tissue factor and the FR and
C regions are derived from one or more human antibodies.

      In accordance with the present invention,
representative nucleic acids encoding CDR-grafted heavy
and light chains were constructed. The CDR-grafted
30 heavy chain comprises a variable region containing FR
regions derived from human antibody KOL and CDRs derived

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from murine monoclonal antibody TF8-5G9 and further
1   comprises a constant region derived from the heavy chain
of human IgG4. The CDR-grafted light chain comprises a
variable region containing FR regions derived from human
antibody REI and CDRs derived from murine monoclonal
5   antibody TF8-5G9 and further comprises a constant region
derived from human IgG4 kappa chain. Nucleic acids
encoding the heavy and light chains were constructed by
assembling the variable regions from synthetic
nucleotides, amplifying the assembled variable regions
10   by PCR, purifying the amplified nucleic acids, and
ligating the nucleic acid encoding the variable region
into a vector containing a nucleic acid encoding the
appropriate human constant region.

    The sequences of representative nucleic acids
15   encoding CDR-grafted heavy and light chains are
presented as nucleotides 1-2360 of SEQ ID NO:15 and
nucleotides 1-759 of SEQ ID NO:20, respectively.

    The nucleic acid sequence encoding a preferred
heavy chain (nucleotides 1-2360 of SEQ ID NO:15) is
20   designated the TF8HCDR20 gene. The nucleic acid
sequence contains the following regions: 5' EcoRI
restriction site (nucleotides 1-6); Kozak sequence
(nucleotides 7-15); start codon and leader sequence
(nucleotides 16-72); CDR-grafted variable region
25   (nucleotides 73-423); human IgG4 CH1 domain (nucleotides
424-717); human IgG4 intron 2 (nucleotides 718-1110);
human IgG4 hinge (nucleotides 1111-1146); human IgG4
intron 3 (nucleotides 1147-1267); human IgG4 CH2 domain
(nucleotides 1268-1594); human IgG4 intron 4
30   (nucleotides 1595-1691); human IgG4 CH3 domain
(nucleotides 1692-2012); 3' untranslated region

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(nucleotides 2013-2354); 3' BamHI end spliced to BclI

1   site of expression vector (nucleotides 2355-2360).

        The nucleic acid sequence encoding a preferred
    light chain gene (nucleotides 1-759 of SEQ ID NO:20) is
    designated the TF8LCDR3 gene. The nucleic acid sequence

5   contains the following regions: 5' EcoRI restriction
    site (nucleotides 1-5); Kozak sequence (nucleotides 6-
    8); start codon and leader sequence (nucleotides 9-68);
    CDR-grafted variable region (nucleotides 69-392); human
    kappa constant region (nucleotides 393-710); 3'

10 untranslated region (nucleotides 711-753); 3' BamHI end
    spliced to BclI site of expression vector (nucleotides
    754-759).

        The foregoing preferred sequences can be
    modified by the ordinarily skilled artisan to take into

15 account degeneracy of the genetic code, and to make
    additions, deletions, and conservative and
    nonconservative substitutions that result in a
    maintenance of the function of the nucleic acid, i.e.
    that it encodes a heavy or light chain of a CDR-grafted

20 antibody capable of inhibiting human tissue factor.
    Restriction sites and sequences that facilitate
    transcription and translation may be altered or
    substituted as necessary depending upon the vector and
    host system chosen for expression.

25   Suitable expression vectors and hosts for
    production of the CDR-grafted antibodies of the present
    invention are known to the ordinarily skilled artisan.
    The expression vectors contain regulatory sequences,
    such as replicons and promoters, capable of directing

30 replication and expression of heterologous nucleic acids
    sequences in a particular host cell. The vectors may

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also contain selection genes, enhancers, signal
1 sequences, ribosome binding sites, RNA splice sites,
polyadenylation sites, transcriptional terminator
sequences, and so on. The vectors may be constructed by
conventional methods well-known in the art, or obtained
5 from commercial sources. The expression vectors
preferably have convenient restriction sites at which
the nucleic acids encoding the antibody chains of the
invention are inserted. Myeloma expression vectors in
which antibody gene expression is driven by the human
10 cytomegalovirus promoter-enhancer or are particularly
preferred.

Expression vectors containing a nucleic acid
encoding the CDR-grafted heavy chain under the control
of a suitable promoter and expression vectors containing
15 a nucleic acid encoding the CDR-grafted light chain
under the control of a suitable promoter are
cotransfected into a suitable host cell. In another
embodiment, nucleic acids encoding both heavy and light
chains are provided in a single vector for transfection
20 of a suitable host cell.

Suitable host cells or cell lines for
expression of the CDR-grafted antibodies of the present
invention include bacterial cells, yeast cells, insect
cells, and mammalian cells such as Chinese hamster ovary
25 (CHO) cells, COS cells, fibroblast cells and myeloid
cells. Mammalian cells are preferred. CHO, COS and
myeloma cells are particularly preferred. Myeloma cells
are preferred for establishing permanent CDR-grafted
antibody producing cell lines. Expression of antibodies
30 in myeloma cells, bacteria, and yeast is reviewed by

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Sandhu (1992) <u>Critical Reviews in Biotechnology</u> <u>12</u>:437.

1   Expression in mammalian cells is reviewed by Owen <u>et</u> <u>al</u>.

Transfection of host cells by the expression vectors containing nucleic acids encoding the CDR-grafted heavy and light chains can be accomplished by

5   methods well-known to one of ordinary skill in the art. Such methods include, for example, calcium chloride transfection, calcium phosphate transfection, lipofection and electroporation. Suitable culture methods and conditions for the production of the CDR-

10   grafted antibodies are likewise well-known in the art. The CDR-grafted antibodies can be purified by conventional methods, including ammonium sulfate precipitation, affinity chromatography, gel electrophoresis, and the like. The ability of the CDR-

15   grafted antibodies to bind to and inhibit human tissue factor can be assessed by the <u>in vitro</u> assays described above.

The CDR-grafted antibodies of the present invention have a variety of utilities. For example, the

20   antibodies are capable of binding to human tissue factor and thus are useful in assays for human tissue factor from body fluid samples, purification of human tissue factor, and so on.

The CDR-grafted antibodies of the present

25   invention are capable of inhibiting human tissue factor. Human tissue factor is well-known to be an essential element in the human coagulation cascade. The ability of the antibodies of the present invention to disrupt the coagulation cascade is demonstrated by <u>in vitro</u>

30   assays in which the antibodies prevent factor X activation. Accordingly, the present antibodies are

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useful in the attenuation of coagulation. The present
1 invention thus provides a method of attenuation of
coagulation comprising administering a therapeutically
effective amount of CDR-grafted antibody capable of
inhibiting human tissue factor to a patient in need of
5 such attenuation.

Numerous thrombotic disorders are
characterized by excessive or inappropriate coagulation
and are effectively treated or prevented by
administration of agents that interfere with the
10 coagulation cascade. Accordingly, the present invention
further provides a method of treatment or prevention of
a thrombotic disorder comprising administering a
therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
15 patient in need of such treatment or prevention. In a
preferred embodiment, the thrombotic disorder is
intravascular coagulation, arterial restenosis or
arteriosclerosis. The antibodies of the invention may be
used in combination with other antibodies or therapeutic
20 agents.

A therapeutically effective amount of the
antibodies of the present invention can be determined by
the ordinarily skilled artisan with regard to the
patient's condition, the condition being treated, the
25 method of administration, and so on. A therapeutically
effective amount is the dosage necessary to alleviate,
eliminate, or prevent the thrombotic disorder as
assessed by conventional parameters. For example, a
therapeutically effective dose of a CDR-grafted antibody
30 of the present invention may be from about 0.1 mg to
about 20 mg per 70 kg of body weight. A preferred

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dosage is about 1.0 mg to about 5 mg per 70 kg of body

1 weight.

A patient in need of such treatment is a patient suffering from a disorder characterized by inappropriate or excessive coagulation, or a patient at

5 risk of such a disorder. For example, anticoagulant therapy is useful to prevent postoperative venous thrombosis, and arterial restenosis following balloon angioplasty.

The CDR-grafted antibodies of the present

10 invention are useful in the same manner as comparable therapeutic agents, and the dosage level is of the same order of magnitude as is generally employed with those comparable therapeutic agents. The present antibodies may be administered in combination with a

15 pharmaceutically acceptable carrier by methods known to one of ordinary skill in the art.

Another embodiment of the present invention is directed to a pharmaceutical composition comprising a least one CDR-grafted antibody capable of inhibiting

20 human tissue factor and further comprising a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying

25 agents, and the like. The use of such media and agents for pharmaceutically active substances is well-known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated.

30 Supplementary active ingredients can also be incorporated into the compositions.

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The antibodies can be administered by well-
1 known routes including oral and parenteral, e.g.,
intravenous, intramuscular, intranasal, intradermal,
subcutaneous, and the like. Parenteral administration
and particularly intravenous administration is
5 preferred. Depending on the route of administration,
the pharmaceutical composition may require protective
coatings.

The pharmaceutical forms suitable for
injectionable use include sterile aqueous solutions or
10 dispersions and sterile powders for the extemporaneous
preparation of sterile injectable solutions or
dispersions. In all cases the ultimate solution form
must be sterile and fluid. Typical carriers include a
solvent or dispersion medium containing, for example,
15 water buffered aqueous solutions (i.e., biocompatible
buffers), ethanol, polyol such as glycerol, propylene
glycol, polyethylene glycol, suitable mixtures thereof,
surfactants or vegetable oils. The antibodies may be
incorporated into liposomes for parenteral
20 administration. Sterilization can be accomplished by an
art-recognized techniques, including but not limited to,
addition of antibacterial or antifungal agents, for
example, paraben, chlorobutanol, phenol, sorbic acid or
thimersal. Further, isotonic agents such as sugars or
25 sodium chloride may be incorporated in the subject
compositions.

Production of sterile injectable solutions
containing the subject antibodies is accomplished by
incorporating these antibodies in the required amount in
30 the appropriate solvent with various ingredients
enumerated above, as required, followed by

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sterilization, preferably filter sterilization. To

1 obtain a sterile powder, the above solutions are vacuum-
dried or freeze-dried as necessary.

The following examples further illustrate the
present invention.

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EXAMPLE 1

1   Isolation and Sequencing of TF8-5G9
               Light Chain (LC) and Heavy Chain (HC)

          Two DNA libraries were generated from oligo
5   (dT)-primed TF8-5G9 hybridoma RNA utilizing standard
     molecular biology procedures as described by Sambrook et
     al. The cDNA was cloned into the Librarian II plasmid
     vector from Invitrogen (San Diego, CA), and the
     libraries were screened for cDNA clones encoding murine
10   IgG HC and LC. A full-length cDNA clone for the heavy
     chain could not be isolated, despite the construction of
     two independent libraries. A random primed TF8-5G9 cDNA
     library was generated to obtain the missing 5' sequence
     of the heavy chain. Consequently, the heavy chain cDNA
15   was in two pieces: a 5' clone of 390 nucleotides and a
     3' clone of 1392 nucleotides. The two HC clones overlap
     by 292 nucleotides.
          The HC and LC clones were completely sequenced
     by the dideoxy chain termination method of Sanger et al.
20   (1977) Proc. Natl. Acad. Sci. USA 74:5463. To verify
     the variable region sequence, sequence was obtained from
     PCR-amplified cDNA that had been synthesized from total
     TF8-5G9 hybridoma RNA. Total TF8-5G9 hybridoma RNA was
     isolated by the guanidinium thiocyanate method of
25   Chrigwin et al. (1970) Biochemistry 18:5294. cDNA was
     synthesized using the Perkin Elmer (Norwalk, CT) GeneAmp
     RNA Polymerase Chain Reaction (PCR) kit with an oligo
     (dT) primer. Components of the same kit were used in
     the PCR to amplify the LC and HC variable regions using
30   primers based on the sequence that had been obtained for
     the cDNA clones. The amplified variable region

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fragments were gel-purified and sequenced according to

1 the method of Tracy <u>et al</u>. (1991) <u>BioTechniques</u> <u>11</u>:68 on
   a Model 373A Applied Biosystems, Inc. (Foster City, CA)
   automated fluorescent DNA sequencer. The sequence for
   TF8-5G9 LC and HC obtained from RNA amplification and

5 the sequence obtained from the cDNA clones agreed. The
   TF8-5G9 HC variable region sequence with protein
   translation is shown in Figure 1 and SEQ ID NO:1, and
   that for the LC is shown in Figure 2 and SEQ ID NO:3.

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EXAMPLE 2

1   Chimeric LC and HC Expression Vector Construction

         In order to test the binding activity of the
CDR-grafted anti-TF LC and HC individually, mouse-human
5   chimeric TF8-5G9 LC and HC were constructed. This
allowed the CDR-grafted LC to be tested for TF binding
ability in combination with the chimeric HC, and the
CDR-grafted HC to be tested in combination with the
chimeric LC.

10   Primers were designed to amplify the TF8-5G9
LC variable region using as template cDNA clones in the
Librarian II vector. The 5' primer was designed with an
EcoRI site while the 3' primer was designed with a NarI
site. PCR was used to amplify the LC variable region,
15   generating a 433 bp fragment with a 5'EcoRI end and
3'NarI end. The fragment included the signal sequence
from the TF8-5G9 LC cDNA clone but incorporated a 2 base
change in the arginine codon immediately following the
ATG start codon. This change retained the arginine
20   residue but made the sequence conform to the Kozak
consensus sequence in order to potentially improve
translation of the LC mRNA. The PCR amplified LC
variable region fragment was digested with EcoRI and
NarI restriction enzymes and purified by electrophoresis
25   on a 2% Nusieve, 1% Seakem agarose gel (FMC Bio
Products, Rockland, ME).

         The DNA was extracted from the gel slice and
purified by the Geneclean (Bio 101, La Jolla, CA)
procedure. The full-length chimeric TF8-5G9 LC gene was
30   generated by cloning this DNA into the EcoRI and NarI
sites of a pSP73 vector (Promega, Madison, WI) which

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contains the human kappa constant region. The gene was
1 isolated from the pSP73 vector by EcoRI digestion and
subcloned into the EcoRI site of the pSG5 mammalian cell
expression vector (Stratagene Cloning Systems, La Jolla,
CA).

5   The chimeric TF8-5G9 HC gene was assembled in
a manner similar to that of the chimeric LC. Since
there was no full-length HC cDNA isolated from the
Librarian II vector cDNA libraries, the HC variable
region fragment that was generated by the PCR from total
10 TF8-5G9 hybridoma cell RNA was used as the template.
Primers which incorporated an EcoRI site at the 5' end
and a SacI site at the 3' end were used in the PCR to
generate a 430 bp fragment which contained the TF8-5G9
HC Kozak sequence, start codon, signal sequence, and
15 variable region. This fragment was digested with the
restriction enzymes EcoRI and SacI, and gel-purified
using the same procedure that was used with the chimeric
LC construction.

        The full-length TF8-5G9 chimeric HC gene was
20 constructed by cloning the variable region fragment into
the EcoRI and SacI sites of the pSG5 expression vector
containing the human IgG4 constant region.

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EXAMPLE 3

1   Design and Construction of the
                 CDR-Grafted Heavy and Light Chain Genes

             The variable region domains of the CDR-grafted
5 HC and LC genes were designed with an EcoRI overhang at
   the 5' end followed by a Kozak sequence to improve
   antibody expression. The leader sequences were derived
   from the heavy and light chains of the murine monoclonal
   antibody B72.3 (Whittle et al. (1987) Protein
10 Engineering 1:499). The 3' end of the variable regions
   were designed to have overhangs which allowed for
   splicing to the appropriate human constant region DNA.
             In the initially designed CDR-grafted TF8-5G9
   heavy and light chains the CDRs were derived from murine
15 TF8-5G9 sequence while the frameworks were derived
   primarily from human antibody sequence. The human
   antibody KOL (Schmidt et al.) was used for the heavy
   chain frameworks, while the human antibody dimer (Epp et
   al.) was used for the light chain frameworks.
20   Several criteria were used to select murine
   framework residues in the design of the TF8-5G9 CDR-
   grafted heavy and light chain variable regions.
   Framework residues which, at a particular position, are
   idiosyncratic to TF8-5G9 were retained as murine
25 sequence with the assumption that they contributed to
   its unique binding characteristics. TF8-5G9 murine
   residues were also retained at framework positions where
   they were in agreement with the human consensus sequence
   but where the corresponding residues in KOL or REI were
30 idiosyncratic. Residues that are part of antibody loop
   canonical structures such as residue 71 (numbering

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according to Kabat <u>et</u> <u>al</u>.) of the heavy and light chains
1 were also retained as murine sequence. Framework
residues that form loops such as residues 26-30 of the
HC were kept as TF8-5G9 murine sequence at positions
were the murine sequence differed from the human.
5 Residues known to directly influence the conformation of
CDRs, such as 48 and 49 immediately preceding CDR2 of
the HC, were also retained as murine sequence.

The amino acid sequence of the variable region
for the initially designed CDR-grafted TF8-5G9 HC,
10 TF8HCDR1, is shown in SEQ ID NO:11. Murine residues
were retained at framework positions 6, 17, 23, 24, 28,
29, 30, 48, 49, 68, 71, 73, 78 88 and 91. The CDR-
grafted HC variable region was attached to a human IgG4
constant region.

15   The amino acid sequence of the variable region
for the initially designed CDR-grafted TF8-5G9 LC,
TF8LCDR1, is shown in SEQ ID NO:12. Murine residues
were retained at framework positions 39, 41, 46 and 105.
The CDR-grafted LC variable region was attached to a
20 human kappa constant region.

The variable region for the CDR-grafted HC and
LC described above were each assembled from 13 synthetic
oligonucleotides which were synthesized by Research
Genetics, Inc., Huntsville, AL. These oligonucleotides
25 ranged in length from 42 to 80 bases, and encoded both
variable region strands. When the 6 complementary
oligonucleotide pairs were annealed, the overhangs
generated were 17 to 24 bases in length. These
oligonucleotide pairs were combined, annealed at their
30 complementary overhangs, and ligated to give the final
full length double-stranded variable regions.

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        The HC variable region oligonucleotides were
1 assembled into a 452 bp fragment which contains a 5'
  EcoRI site and a 3' SacI site. The polymerase chain
  reaction was used to amplify this fragment. The
  resulting amplified DNA was purified on a 2% Nusieve, 1%
5 Seakem agarose gel (FMC). The appropriate size band of
  DNA was excised and the DNA was recovered by the
  Geneclean (Bio 101) procedure. The fragment was then
  digested with EcoRI and SacI, and purified again by the
  Geneclean method. This HC variable region fragment with
10 EcoRI and SacI ends was cloned into the EcoRI and SacI
  sites of the pSport-1 vector (GIBCO-BRL Life
  Technologies, Gaithersburg, MD). DNA from several
  clones was isolated and sequenced to verify proper
  variable region assembly. All clones had unexpected
15 base changes. One clone with the fewest base changes
  (two mismatches at bases 133 and 140) was selected to be
  corrected by site-directed mutagenesis according to
  Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488.
  Briefly, CJ236 (ung-, dut-) competent cells (Invitrogen
20 Corporation, San Diego, CA) were transformed with the
  pSport vector containing the CDR-grafted HC variable
  region with the two base mismatch. Single-stranded,
  uridine-incorporated DNA templates were purified from
  phage following M13 helper phage (Stratagene Cloning
25 Systems) infection of the transformed cells.
  Mutagenesis oligos containing the desired base changes
  were synthesized on an Applied Biosystems Model 380B DNA
  synthesizer. The mutagenesis oligos were annealed to
  the template DNA, and T7 DNA Polymerase and T4 DNA
30 Ligase (MutaGene InVitro Mutagenesis Kit, Bo-Rad
  Laboratories, Richmond, CA) were used to incorporate the

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oligo into a newly synthesized DNA strand. DH5α

1 competent cells (GIBCO-BRL Life Technologies) were
transformed with the double-stranded DNA. The original
uridine-incorporated strand is destroyed while the newly
synthesized strand containing the mutagenesis oligo is

5 replicated. Phagemid DNA was prepared from the
resulting mutagenesis clones and the variable regions
were sequence to identify the clones which had
incorporated the desired changes. The corrected HC
EcoRI/SacI variable region fragment was excised from the

10 pSport vector, purified and ligated into the EcoRI/SacI
sites of a pSG5 vector containing the human IgG4
constant region. This resulted in the generation of a
full-length humanized TF8-5G9 HC gene, TF8HCDR1, in the
pSG5 COS cell expression vector. The vector was

15 designated pSG5TF8HCDR1.

       The CDR-grafted TF8-5G9 LC variable region was
also amplified by the PCR from the assembled synthetic
oligonucleotides into a 433 bp fragment which contained
a 5' EcoRI site and a 3' NarI site. This fragment was

20 purified as described above for the HC, digested with
EcoRI and NarI and purified by the Geneclean procedure.
This fragment was cloned into the EcoRI and NarI sites
of a pSG5 vector which contains the human kappa constant
region. This resulted in the generation of a full-

25 length humanized TF8-5G9 LC gene, TF8LCDR1, in the pSG5
COS cell expression vector. Seven clones were
sequenced, and one was found to have the desired CDR-
grafted LC sequence. The vector was designated
pSQ5TF8LCDR1.

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EXAMPLE 4

1   Expression of the CDR-Grafted
              Heavy and Light Chain Genes in COS Cells

              The transient expression of antibody genes in
5   COS-1 cells provides a rapid and convenient system to
    test antibody gene expression and function. COS-1 cells
    were obtained from the American Type Culture Collection
    (CRL 1650) and cultured in Dulbecco's Modified Eagle
    Medium (DMEM, from GIBCO BRL Life Technologies) with 10%
10 fetal calf serum. The pSG5TF8HCDR1 expression factor
    was cotransfected into COS cells with the pSG5 chimeric
    LC expression vector using the DEAE-Dextran method
    followed by DMSO shock as described by Lopata et al.
    (1984) Nucleic Acids Res. 14:5707. After 4 days of
15 culture, media was harvested from the wells and examined
    for antibody expression levels.
              Antibody levels were determined by an ELISA-
    based assembly assay. Plates were coated with a goat
    anti-human Fc specific antibody. Various dilutions of
20 the COS cell supernatant containing secreted antibody
    were added, incubated for one hour, and washed. A
    horseradish peroxidase-linked goat anti-human kappa
    chain antibody was added, incubated for one hour at room
    temperature, and washed. Substrate for the horseradish
25 peroxidase was added for detection. Antibody levels in
    the COS cell media were found to be nearly undetectable
    for the TF8HCDR1 x chimeric LC. Upon closer examination
    of the TF8HCDR1 variable region sequence, it was found
    that an unexpected base change, which had occurred
30 during the site-directed mutagenesis process described
    in Example 3, introduced a stop codon into framework 4

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of the TF8HCDR1 gene. This substitution was corrected
1 by site-directed mutagenesis as described above.
Thorough sequencing of the variable region confirmed
that the correction was made with no additional changes
introduced. Upon transfection of this corrected
5 TF8HCDR1 gene with the chimeric LC, reasonable
expression levels were obtained.

     COS cells which had been co-transfected with
the CDR-grafted LC expression vector, pSGTF8LCDR1, and
either the chimeric HC or TF8HCDR1, produced antibody at
10 reasonable levels. Antibody levels in COS cell
supernatants ranged from 0.5 μg to 10.0 μg per ml.

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EXAMPLE 5

1   Binding of the CDR-Grafted TF8-5G9 to Tissue Factor

An ELISA was used to determine the ability of
the CDR-grafted TF8-5G9 antibody, TF8HCDR1 x TF8LCDR1,
5 to bind to tissue factor. Tissue factor was immobilized
on a microtiter plate. The test COS cell supernatant,
containing the CDR-grafted antibody, was added to the
well, incubated for one hour at room temperature.
Following three washes with PBS/Tween, a goat anti-human
10 kappa chain polyclonal antibody conjugated to
horseradish peroxidase was added, incubated for one hour
at room temperature and washed. Substrate for the
horseradish peroxidase was added for detection. The
positive control was the TF8-5G9 chimeric antibody. The
15 CDR-grafted TF8-5G9 antibody was able to bind to tissue
factor to a degree comparable to the chimeric TF8-5G9
antibody (Figure 3, solid symbols).
The ability of the humanized antibody to
compete with murine TF8-5G9 for binding to tissue factor
20 was also examined. Varying amounts of COS cell
supernatant containing the test CDR-grafted antibody and
a fixed amount of murine TF8-5G9 were added
simultaneously to wells coated with tissue factor.
Binding was allowed to occur for one hour at room
25 temperature. The wells were washed three times with
PBS/Tween. A goat anti-human kappa chain antibody
conjugated to horseradish peroxidase was added,
incubated for one hour at room temperature and washed.
Substrate for the horseradish peroxidase was added for
30 detection. The positive antibody competed as well as

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the chimeric antibody with murine TF8-5G9 for binding to
1 TF.

            These data indicate that the initially
    designed CDR-grafted antibody, TF8HCDR1 x TF8LCDR1, was
    approximately as active as the chimeric TF8-5G9 in
5   binding to TF and competing with the murine antibody for
    binding to TF.

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EXAMPLE 6

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Construction and Characterization
of Additional CDR-Grafted Heavy Chains

Upon examination of the molecular structure of
5   murine TF8-5G9, framework residues at positions 27, 68,
73 and 78 were found to lie on the antibody surface and
had no discernible contact with the CDRs. These
framework residues were of murine sequence in TF8HCDR1
but were changed to the human KOL sequence in various
10 combinations to generate a series of CDR-grafted heavy
chains with framework residue variations. The changes
were made by the process of site-directed mutagenesis as
described in Example 3. Each CDR-grafted heavy chain
version was expressed in COS cells in combination with
15 the CDR-grafted LC, TF8LCDR1, and tested for its ability
to bind TF and compete with murine TF8-5G9 for binding.
Every version of the CDR-grafted heavy chain in
combination with TF8LCDR1 was shown to bind TF with an
affinity comparable to chimeric TF8-5G9. Every CDR-
20 grafted HC in combination with TF8LCDR1 was able to
compete with murine TF8-5G9 for binding to TF to a
degree comparable to the chimeric antibody.

Changes in sequence from murine to human for
HC framework positions 6, 7, 68, 73 and 78 did not
25 adversely affect the antigen binding ability of the
antibody. The CDR-grafted HC version which had human
sequence at all of these positions, and thus was the
most humanized HC, was TF8HCDR20.

The complete sequence of the TF8HCDR20 gene
30 was determined. The DNA sequence is shown as a 2360 bp
EcoRI/BamHI insert with protein translation in the

35

pEe6TF8HCDR20 expression vector in Figure 4 and SEQ ID
1 NO:15.

       The essential regions of the gene are as

follows:

Nucleotide #	Region
1-6	5' EcoRI restriction site
7-15	Kozak sequence
16-72	Start codon and leader sequence
73-423	CDR-grafted variable region
424-717	Human IgG4 CH1 domain
718-1110	Human IgG4 intron 2
1111-1146	Human IgG4 hinge
1147-1267	Human IgG4 intron 3
1268-1594	Human IgG4 CH2 domain
1595-1691	Human IgG4 intron 4
1692-2012	Human IgG4 CH3 domain
2013-2354	3' untranslated region
2355-2360	3' BamHI end spliced to BclI site of the expression vector

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EXAMPLE 7

Construction and Characterization
of Additional CDR-Grafted Light Chains

The initially designed CDR-grafted LC,
TF8LCDR1, contained four framework residues from the
murine TF8-5G9 sequence. At two of these positions, 39
and 105, the human REI framework sequence is unique to
REI; however, the murine TF8-5G9 LC sequence is in
agreement with the human consensus sequence. The other
two murine framework residues, trp41 and thr46, are
unique to TF8-5G9. Several versions of the CDR-grafted
LC were generated in which the sequence at these four
positions were changed from the murine to the human REI
in various combinations. These changes were made by
site-directed mutagenesis. Each version of the CDR-
grafted LC was expressed in COS cells in combination
with the CDR-grafted HC, TF8HCDR20, and tested for
ability to bind tissue factor and compete with murine
TF8-5G9 for binding. Every version of the CDR-grafted
LC, in combination with TF8HCDR20, was shown to bind TF
with an affinity comparable to TF8-5G9. Also every CDR-
grafted LC version, in combination with TF8HCDR20, was
able to compete with murine TF8-5G9 for binding to TF in
a manner comparable to the chimeric TF8-5G9 control.

Changes in sequence from murine to human for
LC framework positions 39, 41, 46 and 105 did not
adversely effect the ability of the antibody to
recognize antigen. The CDR-grafted LC of choice was
TF8LCDR3, where murine TF8-5G9 sequence was used at
positions 39 and 105 because these are in agreement with

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the human consensus sequence. The preferred CDR-grafted
1 TF8-5G9 antibody is TF8HCDR20 x TF8LCDR3.

      The complete sequence of the TF8LCDR3 gene was
determined and is shown as a 759 bp EcoRI-BamHI insert
with protein translation in the pEe12TF8LCDR3 expression
5 vector in Figure 5 and SEQ ID NO:17. The essential
regions of the gene are as follows:

Nucleotide #	Region
1-5	5' EcoRI restriction site
6-8	Kozak sequence
9-68	Start codon and leader sequence
69-392	CDR-grafted variable region
393-710	Human kappa constant region
711-753	3' untranslated region
754-759	3'BamHI end spliced to BclI site of the expression vector

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EXAMPLE 8

1   CDR-Grafted TF8-5G9 Antibody TF8HCDR20 x TF8LCDR3
                  Inhibits. Human Tissue Factor

         The binding of the CDR-grafted TF8-5G9
5   antibody, TF8HCDR20 x TF8LCDR3, to TF was assessed as
     described in Example 5 and was found to be comparable to
     that of the chimeric TF8-5G9 as illustrated in Figure 6.
     The ability of the CDR-grafted TF8-5G9 to compete with
     the murine antibody for binding to TF is comparable to
10   that of the chimeric TF8-5G9 as shown in Figure 7.
         An _in vitro_ assay was used to measure the
     level of inhibition of factor X activation by the CDR-
     grafted TF8-5G9 antibody. In this assay, TF forms an
     active proteolytic complex with factor VII. This
15   complex then converts factor X to factor Xa by
     proteolysis. The activated Xa enzymatically cleaves a
     substrate, Spectrozyme FXa, which releases a chromogen.
     The level of chromogen, as detected by optical density,
     is an indication of factor X activation due to TF-factor
20   VIIa activity.
         The following reaction mixtures were prepared
     in 12 x 75 mm borosilicate glass tubes.
              25 μl TBS (50 mM Tris, pH 7.4, 150 mM NaCl)
              15 μl 20 mM $CaCl_2$/1% bovine serum albumin
25   (BSA)
              20 μl human placental tissue factor solution
                    (prepared by reconstituting one vial of
                    Thromborel S, Curtin Matheson Scientific
                    #269-338 with 4.0 ml dH_2O and diluting
30   1:10 in TBS)

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30 μl Factor VII (Enzyme Research Labs #HFVII

1   1007 at 237.66 ng/ml in TBS)

30 μl TBS or TF8-5G9 or TF8MCDR20 x TF8LCDR3

at 1.18 μg/ml or as indicated in Fig. 8

The reaction mixtures were incubated at 37°C

5 for ten minutes before the addition of Factor X. (In

some cases the reaction mixture was preincubated for

five minutes before addition of Factor VII or antibody,

followed by a ten minute incubation before addition of

Factor X.) Thirty μl of Factor X solution (Enzyme

10 Research Labs, DHFX 330, 247.38 μg/ml TBS) was added and

the mixture was incubated at 37°C for three minutes.

Factor X activation was terminated by pipetting 40 μg of

reaction mixture into 160 μl of stop buffer (50 mM Tris,

pH 7.4, 100 mM EDTA, 150 mM NaCl) in 96 well microtiter

15 plates. Each tube of reaction mixture was pipetted into

three microtiter wells. Fifty μl of Spectrozyme FXa

substrate (American Diagnostica #222, 1μM/ml TBS) was

added to each well. OD_{405} was read on a Molecular

Devices kinetic plate reader with readings taken every

20 twenty seconds for ten minutes. Factor X activity was

recorded as mOD/minute, and enzyme velocities over the

linear portion of the reaction curve were compared to

determine inhibition of factor X activation by the anti-

TF antibodies.

25   As shown in Figure 8, the CDR-grafted TF8-5G9

antibody is approximately as effective as the murine

TF8-5G9 in inhibiting factor X activation. This

indicates that the CDR-grafted TF8-5G9 is functionally

active.

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EXAMPLE 9

1   Construction of the CDR-Grafted Heavy
              and Light Chain Myeloma Expression Vectors

              For the purpose of establishing a permanent
5   CDR-grafted antibody-producing cell line, the TF8HCDR20
    and TF8LCDR3 genes were subcloned into myeloma cell
    expression vectors. The heavy chain TF8HCDR20 was
    subcloned into the EcoRI and BclI sites of the pEe6hCMV-
    BglII myeloma expression vector described by Stephens et
10 al. (1989) Nucleic Acids Res. 17:7110 to produce
    pEe6TF8HCDR20. The light chain TF8LCDR3 was subcloned
    into the EcoTI and BclI sites of the pEe12 myeloma
    expression vector to produce pEe12TF8LCDR3. The heavy
    and light chain expression vectors are illustrated in
15 Figures 9 and 10, respectively. In both vectors
    antibody gene transcription was driven by the human
    cytomegalovirus (hCMV) promoter-enhancer, which lies
    directly 5' to the multiple cloning site. The
    polyadenylation signal sequence lies 3' to the multiple
20 cloning site and signals the termination of
    transcription. Each vector contains the ß-lactamase
    gene to allow for ampicillin selection in E. coli. The
    pEe12 vector contains a glutamine synthetase cDNA gene
    under the transcriptional control of the SV40 early
25 promoter. Glutamine synthetase allows for myeloma cell
    transfectants to be selected in glutamine-free media.
    Myeloma cells are devoid of glutamine synthetase
    activity and are dependent on a supply of glutamine in
    the culture media. Cells which have been transfected
30 with the pEe12 vector, containing the glutamine

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synthetase gene, are able to synthesize glutamine from

1 glutamate and can survive in the absence of glutamine.

The pEe6TF8HCDR20 expression vector is a 7073 bp
plasmid whose DNA sequence is shown in Figure 4 and SEQ
ID NO:15. The coding regions of the TF8HCDR20 gene are

5 translated. The essential regions of this vector are
described below:

> 1. Nucleotides #1-2360: The TF8HCDR20 CDR-
> grafted HC gene is described in Example
> 6. The HC gene was inserted as an
> EcoRI/BamHI fragment into the EcoRI/BclI
> 10 sites of the pEe6hCMV-BglII vector.
>
> 2. Nucleotides #2361-2593: This region
> encodes the SV40 early gene
> polyadenylation signal (SV40 nucleotides
> 2770-2537), which acts as a
> transcriptional terminator. This
> fragment is flanked by a 5' BclI site and
> 15 a 3' BamHI site. The 3' BamHI end of the
> heavy chain gene was spliced to the 5'
> BclI site of the polyadenylation signal,
> thus eliminating both sites.
>
> 3. Nucleotides #2594-3848: This region is a
> BamHI-BglI fragment from pBR328
> (nucleotides 375-2422) but with a
> 20 deletion between the SaI and AvaI sites
> (pBR328 nucleotides 651-1425) following
> the addition of a SalI linker to the AvaI
> site. This region contains the Col E1
> bacterial origin of replication.
>
> 4. Nucleotides #3849-4327: This is a BglI-
> 25 XmnI fragment site from the ß-lactamase
> gene of pSP64 (Promega Corporation,
> Madison, WI). This gene provides
> ampicillin resistance to bacteria
> transformed with this vector.
>
> 5. Nucleotides #4328-4885: This is an XmnI-
> 30 HindIII fragment of the ColE1 based
> plasmid pCT54 described by Emtage et al.
> (1983) Proc. Natl. Acad. Sci. USA

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80:3671. The HindIII site was converted
to a BglII site by the addition of a
linker following the addition of the hCMV
promoter described below.

6.   Nucleotides #4886-7022: These
     nucleotides encode the Pst-1m fragment of
     human cytomeglovirus (hCMV) strain AD 169
     described by Greenway et al. (1982) Gene
     18:355 containing the region coding for
     the hCMV middle intermediate early
     promoter. This Pst-1m fragment was
     cloned into the HindIII site of pEe6hCMV
     by addition of oligonucleotides of the
     following sequence to either end of the
     fragment:

     5' GTCACCGTCCTTGACACGA 3'

     3' ACGTCAGTGGCAGGAACTGTGCTTCGA 5'

     The resulting 2100 bp fragment was
     inserted such that the promoter directed
     transcription towards the EcoRI site of
     pEe6hCMV. The oligonucleotide above
     served to recreate the complete 5'
     untranslated sequence of the hCMV-MIE
     gene the added irrelevant sequence at the
     very 5' end of the fragment. The HindIII
     site at the 5' end was subsequently
     converted to a BglII site by the addition
     of a further linker.

7.   Nucleotides #7023-7073: The pSP64
     polylinker with the BamHI and SaII sites
     removed.

     The pEe12TF8LCDR3 expression vector is a 7864
bp plasmid whose DNA sequence is shown in Figure 5 and
SEQ ID NO:17. The coding regions of the TF8LCDR3 gene
are translated. The essential regions of this
expression vector are described below:

1.   Nucleotides #1-759: The TF8LCDR3 CDR-
     grafted LC gene is described in Example
     7. The gene was inserted as an

1   EcoRI/BamHI fragment into the EcoRI/BclII
         sites of the pEe12 expression vector.

    2.   Nucleotides #760-3284: These regions of
         pEe12 are identical to the regions
         encoded by nucleotides 2361-4885 of the
         pEe6TF8HCDR20 vector described above
5   (regions #2-5).

    3.   Nucleotides #3285-5736: This region
         encodes the Chinese hamster ovary
         glutamine synthetase cDNA under the
         transcriptional control of the SV40 early
         promoter and followed by the SV40
         polyadenylation and splice signals from
10   the pSV2.dhfr vector described by
         Subramani et al. (1981) Mol. Cell. Biol.
         1:854. The following describes the
         derivation of this region: A 1200 bp
         NaeI-PvuII fragment, containing a
         complete GS coding sequence, was excised
         from the Chinese hamster ovary cDNA clone
15   λGS1.1 described by Hayward et al. (1986)
         Nucleic Acid Res. 14:999. After addition
         of a HindIII linker to the NaeI site and
         a BglII linker to the PvuII site (hence
         destroying the NaeI and PvuII sites), the
         1200 bp fragment was cloned in place of
         DHFR sequences in pSV2.dhfr between the
         HindIII and BglII sites to form pSV2.GS.
20   The single remaining PvuII site in
         pSV2BamGS was converted to a BamHI site
         by addition of an oligonucleotide linker
         to form pSV2BamGS. An EcoRI site in the
         GS cDNA was destroyed by site directed
         mutagenesis without altering the amino
         acid sequence in pSV2BamGS and the
25   HindIII site was destroyed by filling in
         with DNa polymerase I. The 2451 bp BamHI
         fragment from this plasmid, containing
         the complete SV40-GS hybrid transcription
         unit, was excised and inserted at the
         BglII site of pEe6hCMV-BglII site of
         pEe6hCMV-BglII such that transcription
         from the sV40 early promoter proceeds
30   towards the hCMV promoter.

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4.   Nucleotides #5737-7864: This region is
         identical to the hCMV promoter and pSP64
         polylinker encoded by nucleotides 4886-
         7073 of the pEe6TF8HCDR20 vector
         described above (regions 6 and 7).

For the purpose of ensuring that both the
pEe6TF8HCDR20 and peE12TF8LCDR3 vectors co-transfected
myeloma cells, the vectors were joined in linear
concatamers. Both the pEe6TF8HCDR20 and pEe12TF8LCDR3
vectors were digested at the unique _SalI_ site. The _SalI_
linearized pEe6TF8HCDR20 vector was phosphatased at its
5' ends to prohibit ligation of two pEe6TF8HCDR20
vectors onto each other. This phosphatased HC vector
was ligated in a 2:1 molar ratio to the _Sal_ linearized
pEe12TF8LCDR3. The resulting concatamers were most
likely of the following composition:

_Sal_I	_Sal_I	_Sal_I	_Sal_I
pEe6TF8HCDR20	pEe12TF8LCDR3	pEe6TF8HCDR20	
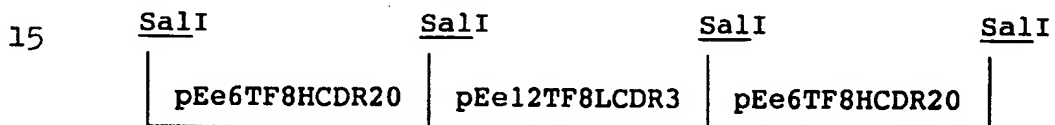
This concatamerized DNA was extracted with phenol and
chloroform, and precipitated with ammonium acetate and
ethanol. The DNA precipitate was resuspended in
distilled water to a concentration of 1 μg/μL and used
to transfect myeloma cells.

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EXAMPLE 10

1   Development of NSO Expression Cell Lines

       Stably transformed cell lines expressing the
humanized TF8-5G9 antibody were prepared by transfecting
5 CDR-grafted heavy and light chain expression vectors
into NSO mouse myeloma cells. Selection of transfected
cells was carried out using the dominant selectable
marker gene, glutamine synthetase (GS).

       The NSO mouse myeloma cell line, obtained from
10 Celltech, Ltd., is a subclone derived from NS-1 and does
not express intracellular light chains. These cells
were cultured in Dulbecco's modified Eagle's medium
(DMEM) with added glutamine and 10% fetal bovine serum
(FBS). To prepare for transfection, the cells were
15 harvested in mid-log phase of the growth cycle,
centrifuged for 5 minutes, washed with phosphate
buffered saline (PBS), centrifuged again, and the cell
pellet was resuspended in 2.2 mL of PBS. The final cell
concentration was 2.18×10^{7} mL. Cells were maintained
20 on ice during the entire procedure.

       The DNA to be transfected (pEe12TF8LCDR3 x
pEe6TF8HCDR20) was prepared as a concatamer as described
in Example 9. The DNA and NSO cells were added to a 0.4
cm BioRad Gene Pulser cuvette in the following order:

25   40 μL (40 μg) DNA concatamer

                320 μL double distilled water

                40 μL 10 x PBS

                400 μL NSO cells (8.72×10^{6} cells)

       Transfection was performed by electroporation
30 following a protocol provided by Celltech, Ltd. In this
procedure, the cells and DNA in PBS buffer were exposed

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to a brief, high voltage pulse of electricity causing
1 transient micropores to form on the cell membrane. DNA
transfer takes place through these openings. To prepare
for electroporation, the suspension of NSO cells and DNA
was gently mixed and incubated on ice for 5 minutes.
5 The cuvette was placed in a BioRad Gene Pulser and given
2 consecutive electrical pulses at settings of 3 μF
(capacitance) and 1.5V (voltage). Following
electroporation, the cuvette was returned to the ice for
5 minutes. The suspension was then diluted in prewarmed
10 growth medium and distributed into seven 96-well plates.
Control plates containing cells electroporated without
DNA were also prepared at the same time to measure the
presence of spontaneous mutants. Plates were placed in
a 37°C incubator with 5% CO_2.
15   Glutamine synthetase, encoded by the GS gene,
is an enzyme that converts glutamate to glutamine. NSO
cells require glutamine for growth due to inadequate
levels of endogenous GS gene expression. In the DNA
concatamer, this gene is located on the pEe12TF8LCDR3
20 vector. Transfected cells which incorporate the GS gene
become glutamine-independent. Cells not integrating the
GS gene into their genome would remain glutamine-
dependent and would not survive in glutamine-free
medium. Approximately 18 hours post electroporation,
25 all plates were fed with glutamine-free selection medium
and returned to the incubator until viable colonies
appeared.
        Approximately 3 weeks after transfection,
distinct macroscopic colonies were observed. These were
30 screened for expression of the intact humanized antibody
using the assembly ELISA as described in Example 5.

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Tissue culture supernatants from wells containing

1 colonies were screened at a 1:10 dilution. Positive
wells showing activity greater than the 25 ng/mL
standard were subcultured and expanded for further
analysis.

5   For selection of high producers, antibody
production was quantitated after a 96 hour growth
period. Tissue culture flasks were seeded with 2×10^5
cells/mL in 10 mL of selection medium and incubated at
37°C, 5% CO_2 for 96 hours. At the end of that time

10 period, an aliquot was taken to determine cell
concentration and antibody titer. Evaluation of
antibody production was calculated as μg/mL and
pg/cell/96 hours. The highest producers from this
transfection were:

15   Cell Line   μg/mL   pg/cell/96 hour
           2B1   26.3   24.3
           3E11   27.6   59.9
           4G6   30.2   41.9

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EXAMPLE 11

1   CDR Grafted Antibody TF8HCDR20 x TF8LCDR3
                    Inhibits Tissue Factor In Vivo

        CDR grafted antibody TF8HCDR20 x TF8LCDR3 was
5 compared to murine antibody TF8-5G9 for its ability to
protect rats from experimentally induced disseminated
intravascular coagulation (DIC). In the DIC model, rats
are challenged with human thromboplastin (a crude tissue
extract containing TF activity), resulting in fibrinogen
10 consumption and death. Pretreatment of rats with anti-
TF antibody was demonstrated to protect rats from
fibrinogen consumption and death as follows.

        Human thromboplastin was prepared as described
in U.S. Patent 5,223,427. Saline control or 30 μ/ml of
15 TF8-5G9 or CDR-grafted antibody was injected through the
tail vein of rats, followed by injection of
thromboplastin equivalent to 200 ng of recombinant TF.
Clotting times were determined at T=0 and T=1 minute as
a measure of fibrinogen concentration. Clotting times
20 are proportional to fibrinogen concentration, with a 60
second clotting time corresponding to an 80% reduction
in fibrinogen concentration. Clotting times of greater
than 60 seconds cannot be accurately measured and were
recorded as 60 seconds.

25   Survivability and clotting times for three
representative studies are shown below.

	Survivors		
	---	---	---
Study	Controls	TF8-5G9	CDR-grafted Ab
1	0/8	5/8	6/8
2	0/8	4/7	7/8
3	0/8	8/8	3/7

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Clotting Times
Controls

	Study #1			Study #2			Study #3	
	T=0	T=1		T=0	T=1		T=0	T=1
	16	>60		18	>60		19	>60
	16	>60		18	>60		21	>60
	16	>60		18	>60		18	>60
	17	>60		18	>60		19	>60
	15	>60		16	>60		18	54
	16	>60		18	>60		18	>60
	16	>60		17	>60		18	>60
	16	>60		17	>60		18	>60

Clotting Times
Murine TF8-5G9

	Study #1			Study #2			Study #3	
	T=0	T=1		T=0	T=1		T=0	T=1
	16	36		18	34		19	28
	15	41		18	36		18	29
	15	33		18	>60		19	29
	15	31		17	>60		18	29
	15	>60		18	50		18	28
	16	>60		17	34		19	40
	16	33		17	34		19	40
	16	33		18	31		19	34
	16	>60					19	>60

Clotting Times
CDR-grafted TF8-5G9

	Study #1			Study #2			Study #3	
	T=0	T=1		T=0	T=1		T=0	T=1
	16	>60		17	>60		21	>60
	16	>60		17	33		18	34
	16	>60		18	32		17	>60
	22	37		18	>60		20	35
	16	32		17	32		17	58
	15	>60		18	31		18	33
	16	>60		17	31		18	31
	16	>60		16	32			

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Twenty-three of the twenty-four control rats
1 had clotting times of greater than 60 seconds indicating
that virtually all untreated rats were consuming more
than 80% of their fibrinogen. Both the CDR-grafted and
murine antibody treated rats had similar clotting times
5 at one minute of 44.5 and 40 seconds. Further, only six
of the murine antibody treated rats and nine of the CDR-
grafted antibody treated rats had clotting times in
excess of 60 seconds. Accordingly, both the murine and
CDR-grafted antibodies were able to neutralize TF and
10 thus protect rats from fibrinogen consumption and death.

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SEQUENCE LISTING

1

(1) GENERAL INFORMATION:

    (i) APPLICANT: Joliffe, Linda K.
                   Zivin, Robert A.
5   Pulito, Virginia L.

   (ii) TITLE OF INVENTION: CDR-GRAFTED ANTI-TISSUE FACTOR
        ANTIBODIES AND METHODS OF USE THEREOF

 (iii) NUMBER OF SEQUENCES: 20

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      (F) ZIP: 11530

   (v) COMPUTER READABLE FORM:
      (A) MEDIUM TYPE: Floppy disk
      (B) COMPUTER: IBM PC compatible
15   (C) OPERATING SYSTEM: PC-DOS/MS-DOS
      (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

  (vi) CURRENT APPLICATION DATA:
      (A) APPLICATION NUMBER:
      (B) FILING DATE: 07-JUN-1995
      (C) CLASSIFICATION:

(viii)-ATTORNEY/AGENT INFORMATION:
20   (A) NAME: DiGiglio, Frank S.
      (B) REGISTRATION NUMBER: 31,346
      (C) REFERENCE/DOCKET NUMBER: 9598

  (ix) TELECOMMUNICATION INFORMATION:
      (A) TELEPHONE: (516) 742-4343
      (B) TELEFAX: (516) 742-4366
      (C) TELEX: 230 901 SANS UR

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(2) INFORMATION FOR SEQ ID NO:1:

1   (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 1489 base pairs
                    (B) TYPE: nucleic acid
                    (C) STRANDEDNESS: double
                    (D) TOPOLOGY: linear

5   (ii) MOLECULE TYPE: DNA (genomic)

           (ix) FEATURE:
                    (A) NAME/KEY: CDS
                    (B) LOCATION: 11..1391

           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
     GGTCCTTACA ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG          49
10              Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val
                 1               5                  10

     GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG         97
     Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu
          15                  20                  25

     CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC        145
     Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly
15   30                  35                  40                  45

     TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA        193
     Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu
                     50                  55                  60

     CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT        241
     Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr
                 65                  70                  75

20   ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA        289
     Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr
                 80                  85                  90

     TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC        337
     Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp
          95                  100                 105

25   ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC        385
     Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr
     110                 115                 120                 125
```

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```
      TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC     433
   1  Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro
                      130             135             140

      CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC     481
      Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser
                  145             150             155

      ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG     529
   5  Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val
                  160             165             170

      ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC     577
      Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe
                  175             180             185

      CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT     625
  10  Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr
      190             195             200             205

      GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC     673
      Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala
                  210             215             220

      CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT     721
      His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp
                  225             230             235
  15
      TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC     769
      Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val
                  240             245             250

      TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT     817
      Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr
                  255             260             265

  20  CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG     865
      Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu
      270             275             280             285

      GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG     913
      Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln
                  290             295             300

      ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT     961
  25  Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser
                  305             310             315
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```
     GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA    1009
     Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys
 1       320                 325                 330

     TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC    1057
     Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile
         335                 340                 345

     TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA    1105
 5   Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro
     350                 355                 360                 365

     CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG AAC TGC ATG    1153
     Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met
                     370                 375                 380

     ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT    1201
     Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn
10           385                 390                 395

     GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA    1249
     Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr
             400                 405                 410

     GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC    1297
     Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn
         415                 420                 425

15   TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG    1345
     Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu
     430                 435                 440                 445

     CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA T      1391
     His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
                     450                 455                 460

20   GATCCCAGTG TCCTTGGAGC CCTCTGGTCC TACAGGACTC TGACACCTAC CTCCACCCCT  1451

     CCCTGTATAA ATAAAGCACC CAGCACTGCC TTGGACCC                         1489
```

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:

25
        (A) LENGTH: 460 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly
 1               5                  10                  15

Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
            20                  25                  30

Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile
        35                  40                  45

Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
    50                  55                  60

Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp
 65                  70                  75                  80

Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn
                85                  90                  95

Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln
        115                 120                 125

Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val
    130                 135                 140

Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr
145                 150                 155                 160

Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr
                165                 170                 175

Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val
            180                 185                 190

Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
        195                 200                 205

Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala
    210                 215                 220
```

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      Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys
      225             230             235             240
  1
      Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe
                      245             250             255

      Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val
              260             265             270

  5   Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe
              275             280             285

      Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro
          290             295             300

      Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
      305             310             315             320

 10   Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val
                      325             330             335

      Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
              340             345             350

      Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys
              355             360             365

 15   Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met Ile Thr Asp
          370             375             380

      Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro
      385             390             395             400

      Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser
              405             410             415

 20   Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala
              420             425             430

      Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His
              435             440             445

      His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
          450             455             460

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```

(2) INFORMATION FOR SEQ ID NO:3:

1   (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 937 base pairs
             (B) TYPE: nucleic acid
             (C) STRANDEDNESS: double
             (D) TOPOLOGY: linear

5   (ii) MOLECULE TYPE: peptide

         (ix) FEATURE:
             (A) NAME/KEY: CDS
             (B) LOCATION: 5..706

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
      GGAC ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT        49
10         Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe
            1               5                  10                  15

      CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG        97
      Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met
                      20              25                  30

      TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG       145
15    Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln
                  35                  40                  45

      GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT       193
      Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser
              50                  55                  60

      CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA       241
      Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro
          65                  70                  75
20
      TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC       289
      Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile
      80                  85                  90                  95

      AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT       337
      Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His
                      100                 105                 110

25    GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC       385
      Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn
                  115  .               120                 125
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```
    AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG        433
    Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
1       130             135             140

    CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC        481
    Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
        145             150             155

    TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA        529
5   Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
    160             165             170             175

    CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC        577
    Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
                180             185             190

    ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA        625
    Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
10              195             200             205

    CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA        673
    Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
        210             215             220

    CCC AAT GTC AAG AGC TTC AAC AAG AAT GAG TGT TAGAGACAAA GGTCCTGAGA       726
    Pro Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
        225             230
15
    CGCCACCACC AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC       786

    CACAAGCGAC CTACCACTGT TGCGGTGCTC CAAACCTCCT CCCCACCTCC TTCTCCTCCT       846

    CCTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTTGCAGA AAATATTCAA TAAAGTGAGT       906

    CTTTGCACTT GAAAAAAAAA AAAAAAAAAA A                                      937
20

    (2) INFORMATION FOR SEQ ID NO:4:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 234 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

25      (ii) MOLECULE TYPE: protein
```

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
 1  Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe Pro
     1               5                  10                  15

    Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
                 20                  25                  30

 5  Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
             35                  40                  45

    Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser Pro
         50                  55                  60

    Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser
     65                  70                  75                  80

10  Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
                     85                  90                  95

    Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His Gly
                100                 105                 110

    Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn Arg
                115                 120                 125

15  Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
            130                 135                 140

    Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
    145                 150                 155                 160

    Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
                    165                 170                 175

20  Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
                180                 185                 190

    Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
                195                 200                 205

    His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
        210                 215                 220

25  Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
    225                 230
```

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(2) INFORMATION FOR SEQ ID NO:5:

1   (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 5 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: double
              (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide
5

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

        Asp Asp Tyr Met His
        1   5

10 (2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 17 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: double
              (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide
15

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

        Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Lys Pro Lys Phe Gln
        1   5   10   15

        Gly

20

    (2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 8 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: double
              (D) TOPOLOGY: linear
25
        (ii) MOLECULE TYPE: peptide

30

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

1 Asp Asn Ser Tyr Tyr Phe Asp Tyr
   1   5

(2) INFORMATION FOR SEQ ID NO:8:

5 (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 11 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: double
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

  Lys Ala Ser Gln Asp Ile Arg Lys Tyr Leu Asn
  1   5   10

(2) INFORMATION FOR SEQ ID NO:9:

15 (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 7 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: double
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

  Tyr Ala Thr Ser Leu Ala Asp
  1   5

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(2) INFORMATION FOR SEQ ID NO:10:

1
    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 9 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: double
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide
5

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Leu Gln His Gly Glu Ser Pro Tyr Thr
1   5

10 (2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 117 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide
15

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1   5   10   15

Leu Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20   25   30
20

Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35   40   45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
50   55   60

Gln Gly Arg Phe Ser Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Phe
65   70   75   80
25

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        Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                    85              90                  95
1
        Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
                    100             105                 110

        Val Thr Val Ser Ser
                    115

5

    (2) INFORMATION FOR SEQ ID NO:12:

        (i)  SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 108 amino acids
             (B) TYPE: amino acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear
10
        (ii) MOLECULE TYPE: peptide


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

        Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
        1               5               10                  15
15      Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
                    20              25                  30

        Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ala Pro Lys Thr Leu Ile
                35              40              45

        Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
            50              55              60

20      Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
        65              70              75                  80

        Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
                    85              90                  95

        Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
                    100             105

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(2) INFORMATION FOR SEQ ID NO:13:

1   (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 117 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

5

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1   5   10   15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
        20   25   30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
      35   40   45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
      50   55   60

Gln Gly Arg Phe Thr Ile Ser Ala Asp Asn Ser Lys Asn Thr Leu Phe
65   70   75   80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
        85   90   95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
        100   105   110

Val Thr Val Ser Ser
      115

20

(2) INFORMATION FOR SEQ ID NO:14:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 108 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
25   (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
            20   25   30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
            35   40   45

Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
            85   90   95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
            100   105

(2) INFORMATION FOR SEQ ID NO:15:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 7073 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 61..717

        (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 1111..1146

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        (ix) FEATURE:
              (A) NAME/KEY: CDS
   1          (B) LOCATION: 1268..1594

        (ix) FEATURE:
              (A) NAME/KEY: CDS
              (B) LOCATION: 1692..2012


   5    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

        GAATTCGCCT CCACCATGGA ATGGAGCTGG GTCTTTCTCT TCTTCTTGTC AGTAACTACA        60

        GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA GGA GGA GTA GTA        108
        Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val
         1               5                   10                  15

        CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT AGT GGA TTC AAT        156
   10   Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
                     20                  25                  30

        ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT CCT GGA AAA GGA        204
        Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
             35                  40                  45

        CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT AAC ACG ATA TAT        252
        Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
   15        50                  55                  60

        GAT CCC AAG TTC CAA GGA AGA TTC ATA ATT TCT GCA GAC AAC TCT AAG        300
        Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
         65                  70                  75                  80

        AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT GAG GAT ACA GCA        348
        Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
                         85                  90                  95
   20
        GTC TAC TTT TGT GCT AGA GAT AAC AGT TAT TAC TTC GAC TAC TGG GGC        396
        Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
                        100                 105                 110

        CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC AAG GGC CCA TCC        444
        Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
                    115                 120                 125

   25   GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC        492
        Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
                130                 135                 140
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    GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG    540
    Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
1   145                 150                 155                 160

    TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT    588
    Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                    165                 170                 175

    GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG    636
5   Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
                    180                 185                 190

    CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC    684
    Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
                195                 200                 205

    AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGTGAGAGGC CAGCACAGGG   737
    Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
10      210                 215

    CAGGGAGGGT GTCTGCTGGA AGCCAGGCTC AGCCCTCCTG CCTGGACGCA CCCCGGCTGT   797

    GCAGCCCCAG CCCAGGGCAG CAAGGCATGC CCCATCTGTC TCCTCACCCG GAGGCCTCTG   857

    ACCACCCCAC TCATGCTCAG GGAGAGGGTC TTCTGGATTT TTCCACCAGG CTCCGGGCAG   917

    CCACAGGCTG GATGCCCCTA CCCCAGGCCC TGCGCATACA GGGGCAGGTG CTGCGCTCAG   977
15
    ACCTGCCAAG AGCCATATCC GGGAGGACCC TGCCCCTGAC CTAAGCCCAC CCCAAAGGCC  1037

    AAACTCTCCA CTCCCTCAGC TCAGACACCT TCTCTCCTCC CAGATTCGAG TAACTCCCAA  1097

    TCTTCTCTCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA     1146
                Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
                1                 5                 10

20  GGTAAGCCAA CCCAGGCCTC GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC  1206

    TGCATCCAGG GACAGGCCCC AGCCGGGTGC TGACGCATCC ACCTCCATCT CTTCCTCAGC  1266

    A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA      1312
      Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
      1                 5                 10                15

    CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG   1360
25  Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
                    20                  25                  30
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   GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC    1408
   Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
1           35              40              45

   GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG    1456
   Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
           50              55              60

   CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC ATG CAC    1504
5  Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His
           65              70              75

   CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA    1552
   Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
   80              85              90              95

   GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA            1594
   Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
10              100             105

   GGTGGGACCC ACGGGGTGCG AGGGCCACAT GGACAGAGGT CAGCTCGGCC CACCCTCTGC   1654

   CCTGGGAGTG ACCGCTGTGC CAACCTCTGT CCCTACA GGG CAG CCC CGA GAG CCA    1709
                                            Gly Gln Pro Arg Glu Pro
                                             1               5

   CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG    1757
15 Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
               10              15              20

   GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC    1805
   Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
               25              30              35

   GTG GAG TGG GAG AGT AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG    1853
   Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
20      40              45              50

   CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA    1901
   Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
   55              60              65              70

   ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA GTC TCC    1949
   Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Val Ser
               75              80              85
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          GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC      1997
          Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
      1              90              95                  100

          CTG TCT CTG GGT AAA TGAGTGCCAG GGCCGGCAAG CCCCCGCTCC CCGGGCTCTC      2052
          Leu Ser Leu Gly Lys
                   105

      5   GGGGTCGCGC GAGGATGCTT GGCACGTACC CCGTCTACAT ACTTCCCAGG CACCCAGCAT      2112

          GGAAATAAAG CACCCACCAC TGCCCTGGGC CCCTGTGAGA CTGTGATGGT TCTTTCCACG      2172

          GGTCAGGCCG AGTCTGAGGC CTGAGTGACA TGAGGGAGGC AGAGCGGGTC CCACTGTCCC      2232

          CACACTGGCC CAGGCTGTGC AGGTGTGCCT GGGCCACCTA GGGTGGGGCT CAGCCAGGGG      2292

          CTGCCCTCGG CAGGGTGGGG GATTTGCCAG CGTGGCCCTC CCTCCAGCAG CAGGACTCTA      2352

     10   GAGGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTTGCTTTAA AAAACCTCCC      2412

          ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTTGTTA ACTTGTTTAT      2472

          TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT      2532

          TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG      2592

     15   GATCCTCTAC GCCGGACGCA TCGTGGCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG      2652

          CGCCTATATC GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG      2712

          CGCTTGTTTC GGCGTGGGTA TGGTGGCAGG CCCGTGGCCG GGGGACTGTT GGGCGCCATC      2772

          TCCTTGCATG CACCATTCCT TGCGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGGC      2832

          TGCTTCCTAA TGCAGGAGTC GCATAAGGGA GAGCGTCGAC CTCGGGCCGC GTTGCTGGCG      2892

     20   TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG      2952

          TGGCGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG      3012

          CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA      3072

          AGCGTGGCGC TTTCTCAATG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC      3132

          TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT      3192

     25   AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT      3252
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     GGTAACAGGA TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG    3312
  1  CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT    3372
     ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT    3432
     GGTTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT    3492
     TTGATCTTTT CTACGGGGTC TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG    3552
  5  GTCATGAGAT TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT    3612
     AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT    3672
     GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC    3732
     GTGTAGATAA CTACGATACG GAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG    3792
 10  CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC    3852
     GAGCGCAGAA GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG    3912
     GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA    3972
     GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCATCAT TCAGCTCCGG TTCCCAACGA    4032
     TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT    4092
 15  CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG    4152
     CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA    4212
     ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAACA    4272
     CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT    4332
 20  TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT    4392
     CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA    4452
     ACAGGAAGGC AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC    4512
     ATACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA    4572
     TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA    4632
 25  AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TAAAAATAGG    4692
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    CGTATCACGA GGCCCTGATG GCTCTTTGCG GCACCCATCG TTCGTAATGT TCCGTGGCAC   4752
1   CGACGACAAC CCTCAAGAGA AAATGTAATC ACACTGGCTC ACCTTCGGGT GGGCCTTTCT   4812
    GCGTTTATAA GGAGACACTT TATGTTTAAG AAGGTTGGTA AATTCCTTGC GGCTTTGGCA   4872
    GCCAAGCTAG AGATCTCTAG CTTCGTGTCA AGGACGGTGA CTGCAGTGAA TAATAAAATG   4932
5   TGTGTTTGTC CGAAATACGC GTTTTGAGAT TTCTGTCGCC GACTAAATTC ATGTCGCGCG   4992
    ATAGTGGTGT TTATCGCCGA TAGAGATGGC GATATTGGAA AAATCGATAT TTGAAAATAT   5052
    GGCATATTGA AAATGTCGCC GATGTGAGTT TCTGTGTAAC TGATATCGCC ATTTTTCCAA   5112
    AAGTGATTTT TGGGCATACG CGATATCTGG CGATAGCGCT TATATCGTTT ACGGGGGATG   5172
    GCGATAGACG ACTTTGGTGA CTTGGGCGAT TCTGTGTGTC GCAAATATCG CAGTTTCGAT   5232
10  ATAGGTGACA GACGATATGA GGCTATATCG CCGATAGAGG CGACATCAAG CTGGCACATG   5292
    GCCAATGCAT ATCGATCTAT ACATTGAATC AATATTGGCC ATTAGCCATA TTATTCATTG   5352
    GTTATATAGC ATAAATCAAT ATTGGCTATT GGCCATTGCA TACGTTGTAT CCATATCATA   5412
    ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA   5472
    CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT ATGGAGTTCC   5532
15  GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCGCCCAT   5592
    TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC   5652
    AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC   5712
    CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT   5772
20  ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA   5832
    CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG   5892
    GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC   5952
    GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG   6012
25  TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC   6072
    GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA CCGATCCAGC CTCCGCGGCC   6132
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    GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGACGTAAGT ACCGCCTATA    6192
1   GAGTCTATAG GCCCACCCCC TTGGCTTCTT ATGCATGCTA TACTGTTTTT GGCTTGGGGT    6252
    CTATACACCC CCGCTTCCTC ATGTTATAGG TGATGGTATA GCTTAGCCTA TAGGTGTGGG    6312
    TTATTGACCA TTATTGACCA CTCCCCTATT GGTGACGATA CTTTCCATTA CTAATCCATA    6372
    ACATGGCTCT TTGCCACAAC TCTCTTTATT GGCTATATGC CAATACACTG TCCTTCAGAG    6432
5   ACTGACACGG ACTCTGTATT TTTACAGGAT GGGGTCTCAT TTATTATTTA CAAATTCACA    6492
    TATACAACAC CACCGTCCCC AGTGCCCGCA GTTTTTATTA AACATAACGT GGGATCTCCA    6552
    CGCGAATCTC GGGTACGTGT TCCGGACATG GGCTCTTCTC CGGTAGCGGC GGAGCTTCTA    6612
    CATCCGAGCC CTGCTCCCAT CCCTCCAGCG ACTCATGGTC GCTCGGCAGC TCCTTGCTCC    6672
10  TAACAGTGGA GGCCAGACTT AGGCACAGCA CGATGCCCAC CACCACCAGT GTGCCGCACA    6732
    AGGCCGTGGC GGTAGGGTAT GTGTCTGAAA ATGAGCTCGG GGAGCGGGCT TGCACCGCTG    6792
    ACGCATTTGG AAGACTTAAG GCAGCGGCAG AAGAAGATGC AGGCAGCTGA GTTGTTGTGT    6852
    TCTGATAAGA GTCAGAGGTA ACTCCCGTTG CGGTGCTGTT AACGGTGGAG GGCAGTGTAG    6912
    TCTGAGCAGT ACTCGTTGCT GCCGCGCGCG CCACCAGACA TAATAGCTGA CAGACTAACA    6972
15  GACTGTTCCT TTCCATGGGT CTTTTCTGCA GTCACCGTCC TTGACACGAA GCTTGGGCTG    7032
    CAGGTCGATC GACTCTAGAG GATCGATCCC CGGGCGAGCT C                        7073
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(2) INFORMATION FOR SEQ ID NO:16:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 219 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: protein
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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1   Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Val Val
    1               5                   10                  15

    Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
                20              25              30

5   Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
                35              40              45

    Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
        50              55                  60

    Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
    65              70              75              80

10  Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
                85                  90                  95

    Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
            100             105             110

    Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115             120             125

15  Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
        130             135             140

    Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
    145             150             155             160

    Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165             170             175

20  Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
                180             185             190

    Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
        195             200             205

    Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
        210             215
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(2) INFORMATION FOR SEQ ID NO:17:

1　　　　　(i) SEQUENCE CHARACTERISTICS:
　　　　　　　　　(A) LENGTH: 12 amino acids
　　　　　　　　　(B) TYPE: amino acid
　　　　　　　　　(D) TOPOLOGY: linear

　　　　　(ii) MOLECULE TYPE: protein

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　　　　　(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
　1　　　　　　　　5　　　　　　　　　　10

(2) INFORMATION FOR SEQ ID NO:18:
10
　　　　　(i) SEQUENCE CHARACTERISTICS:
　　　　　　　　　(A) LENGTH: 109 amino acids
　　　　　　　　　(B) TYPE: amino acid
　　　　　　　　　(D) TOPOLOGY: linear

　　　　　(ii) MOLECULE TYPE: protein

15　　　　　(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
　1　　　　　　　5　　　　　　　　　10　　　　　　　　　15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
　　　　　　　　20　　　　　　　　　25　　　　　　　　　30

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
20　　　　　　35　　　　　　　　　40　　　　　　　　　45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
　　　　　　50　　　　　　　　　55　　　　　　　　　60

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His Gln
　65　　　　　　　　70　　　　　　　　　75　　　　　　　　　80

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
25　　　　　　　　85　　　　　　　　　90　　　　　　　　　95

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Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
            100   105
1

(2) INFORMATION FOR SEQ ID NO:19:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 107 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
 1   5   10   15

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
            20   25   30

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
        35   40   45

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
    50   55   60

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
65   70   75   80

Asn Val Phe Ser Val Ser Val Met His Glu Ala Leu His Asn His Tyr
            85   90   95

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
            100   105

(2) INFORMATION FOR SEQ ID NO:20:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 7864 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: peptide

1   (ix) FEATURE:
                  (A) NAME/KEY: CDS
                  (B) LOCATION: 9..711

           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

5   AATTCACCAT GGGTGTGCCA ACTCAGGTAT TAGGATTACT GCTGCTGTGG CTTACAGATG   60

    CAAGATGTGA TATCCAAATG ACACAATCTC CTTCTTCTCT AAGTGCTTCT GTCGGAGATA   120

    GAGTAACAAT TACATGTAAG GCGAGTCAGG ACATTAGAAA GTATTTAAAC TGGTATCAGC   180

    AAAAACCTGG GAAGGCTCCT AAGCTACTGA TTTATTATGC AACAAGTTTG GCAGATGGAG   240

    TACCTTCTAG ATTTTCTGGT TCTGGCTCTG GAACAGACTA CACATTCACA ATTTCTTCTC   300
10
    TCCAACCTGA GGACATTGCT ACATACTACT GCCTACAACA TGGTGAGAGT CCGTATACAT   360

    TTGGACAAGG AACAAAACTA GAGATCACAA GAACTGTTGC GGCGCCGTCT GTCTTCATCT   420

    TCCCGCCATC TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA   480

    ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA   540

15 ACTCCCAGGA GAGTGTCACA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA   600

    CCCTGACGCT GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC   660

    ATCAGGGCCT GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT TAGAGGGAGA   720

    AGTGCCCCCA CCTGCTCCTC AGTTCCAGCC TGGGGATCAT AATCAGCCAT ACCACATTTG   780

    TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA   840
20
    TGAATGCAAT TGTTGTTGTT AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA   900

    ATAGCATCAC AAATTTCACA AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT   960

    CCAAACTCAT CAATGTATCT TATCATGTCT GGATCCTCTA CGCCGGACGC ATCGTGGCCG   1020

    GCATCACCGG CGCCACAGGT GCGGTTGCTG CGCCTATAT CGCCGACATC ACCGATGGGG   1080

25 AAGATCGGGC TCGCCACTTC GGGCTCATGA GCGCTTGTTT CGGCGTGGGT ATGGTGGCAG   1140

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      GCCCGTGGCC GGGGGACTGT TGGGCGCCAT CTCCTTGCAT GCACCATTCC TTGCGGCGGC    1200
  1   GGTGCTCAAC GGCCTCAACC TACTACTGGG CTGCTTCCTA ATGCAGGAGT CGCATAAGGG    1260
      AGAGCGTCGA CCTCGGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA    1320
      GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA    1380
  5   CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC    1440
      CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG    1500
      TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC    1560
      CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCÀ ACCCGGTAAG    1620
      ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT    1680
 10   AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT    1740
      ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG    1800
      ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC    1860
      GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA    1920
 15   GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC    1980
      CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC    2040
      TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT    2100
      TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT    2160
      ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT    2220
 20   ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC    2280
      CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA    2340
      TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG    2400
      TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT    2460
      GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC    2520
 25   AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT    2580
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      AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG   2640
 1    GCGACCGAGT TGCTCTTGCC CGGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC   2700
      TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC   2760
      GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT   2820
      TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG   2880
 5    AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG   2940
      CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA   3000
      ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT   3060
      TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTGAT GGCTCTTTGC   3120
10    GGCACCCATC GTTCGTAATG TTCCGTGGCA CCGAGGACAA CCCTCAAGAG AAAATGTAAT   3180
      CACACTGGCT CACCTTCGGG TGGGCCTTTC TGCGTTTATA AGGAGACACT TTATGTTTAA   3240
      GAAGGTTGGT AAATTCCTTG CGGCTTTGGC AGCCAAGCTA GAGATCCGGC TGTGGAATGT   3300
      GTGTCAGTTA GGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT   3360
      GCATCTCAAT TAGTCAGCAA CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC   3420
15    TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC   3480
      CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG   3540
      AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG   3600
      GCTTTTGCAA AAAGCTAGCT TGGGGCCACC GCTCAGAGCA CCTTCCACCA TGGCCACCTC   3660
20    AGCAAGTTCC CACTTGAACA AAAACATCAA GCAAATGTAC TTGTGCCTGC CCAGGGTGA    3720
      GAAAGTCCAA GCCATGTATA TCTGGGTTGA TGGTACTGGA GAAGGACTGC GCTGCAAAAC   3780
      CCGCACCCTG GACTGTGAGC CCAAGTGTGT AGAAGAGTTA CCTGAGTGGA ATTTTGATGG   3840
      CTCTAGTACC TTTCAGTCTG AGGGCTCCAA CAGTGACATG TATCTCAGCC CTGTTGCCAT   3900
      GTTTCGGGAC CCCTTCCGCA GAGATCCCAA CAAGCTGGTG TTCTGTGAAG TTTTCAAGTA   3960
25    CAACCGGAAG CCTGCAGAGA CCAATTTAAG GCACTCGTGT AAACGGATAA TGGACATGGT   4020
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    GAGCAACCAG CACCCCTGGT TTGGAATGGA ACAGGAGTAT ACTCTGATGG GAACAGATGG    4080
1   GCACCCTTTT GGTTGGCCTT CCAATGGCTT TCCTGGGCCC CAAGGTCCGT ATTACTGTGG    4140
    TGTGGGCGCA GACAAAGCCT ATGGCAGGGA TATCGTGGAG GCTCACTACC GCGCCTGCTT    4200
    GTATGCTGGG GTCAAGATTA CAGGAACAAA TGCTGAGGTC ATGCCTGCCC AGTGGGAACT    4260
5   CCAAATAGGA CCCTGTGAAG GAATCCGCAT GGGAGATCAT CTCTGGGTGG CCCGTTTCAT    4320
    CTTNCATCGA GTATGTGAAG ACTTTGGGGT AATAGCAACC TTTGACCCCA AGCCCATTCC    4380
    TGGGAACTGG AATGGTGCAG GCTGCCATAC CAACTTTAGC ACCAAGGCCA TGCGGGAGGA    4440
    GAATGGTCTG AAGCACATCG AGGAGGCCAT CGAGAAACTA AGCAAGCGGC ACCGGTACCA    4500
    CATTCGAGCC TACGATCCCA AGGGGGGCCT GGACAATGCC CGTGGTCTGA CTGGGTTCCA    4560
10  CGAAACGTCC AACATCAACG ACTTTTCTGC TGGTGTCGCC AATCGCAGTG CCAGCATCCG    4620
    CATTCCCCCG ACTGTCGGCC AGGAGAAGAA AGGTTACTTT GAAGACCGCG GCCCCTCTGC    4680
    CAATTGTGAC CCCTTTGCAG TGACAGAAGC CATCGTCCGC ACATGCCTTC TCAATGAGAC    4740
    TGGCCACGAG CCCTTCCAAT ACAAAAACTA ATTAGACTTT GAGTGATCTT GAGCCTTTCC    4800
15  TAGTTCATCC CACCCCGCCC CAGAGAGATC TTTGTGAAGG AACCTTACTT CTGTGGTGTG    4860
    ACATAATTGG ACAAACTACC TACAGAGATT TAAAGCTCTA AGGTAAATAT AAAATTTTTA    4920
    AGTGTATAAT GTGTTAAACT ACTGATTCTA ATTGTTTGTG TATTTTAGAT TCCAACCTAT    4980
    GGAACTGATG AATGGGAGCA GTGGTGGAAT GCCTTTAATG AGGAAAACCT GTTTTGCTCA    5040
    GAAGAAATGC CATCTAGTGA TGATGAGGCT ACTGCTGACT CTCAACATTC TACTCCTCCA    5100
20  AAAAAGAAGA GAAAGGTAGA ACACCCCAAG GACTTTCCTT CAGAATTGCT AAGTTTTTTG    5160
    AGTCATGCTG TGTTTAGTAA TAGAACTCTT GCTTGCTTTG CTATTTACAC CACAAAGGAA    5220
    AAAGCTGCAC TGCTATACAA GAAAATTATG GAAAAATATT CTGTAACCTT TATAAGTAGG    5280
    CATAACAGTT ATAATCATAA CATACTGTTT TTTCTTACTC CACACAGGCA TAGAGTGTCT    5340
25  GCTATTAATA ACTATGCTCA AAAATTGTGT ACCTTTAGCT TTTTAATTTG TAAAGGGGTT    5400
    AATAAGGAAT ATTTGATGTA TAGTGCCTAG ACTAGAGATC ATAATCAGCC ATACCACATT    5460
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      TGTAGAGGTT TTACTTCCTT TAAAAAACCT CCCACACCTC CCCCTGAACC TGAAACATAA    5520

  1   AATGAATGCA ATTGTTGTTG TTAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG    5580

      CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT    5640

      GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCTCT AGCTTCGTGT CAAGGACGGT    5700

      GACTGCAGTG AATAATAAAA TGTGTGTTTG TCCGAAATAC GCGTTTTGAG ATTTCTGTCG    5760

  5   CCTACTAAAT TCATGTCGCG CGATAGTGGT GTTTATCGCC GATAGAGATG GCGATATTGG    5820

      AAAAATCGAT ATTTGAAAAT ATGGCATATT GAAAATGTCG CCGATGTGAG TTTCTGTGTA    5880

      ACTGATATCG CCATTTTTCC AAAAGTGATT TTTGGGCATA CGCGATATCT GGCGATAGCG    5940

      CTTATATCGT TTACGGGGGA TGGCGATAGA CGACTTTGGT GACTTGGGCG ATTCTGTGTG    6000

 10   TCGCAAATAT CGCAGTTTCG ATATAGGTGA CAGACGATAT GAGGCTATAT CGCCGATAGA    6060

      GGCGACATCA AGCTGGCACA TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG    6120

      CCATTAGCCA TATTATTCAT TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG    6180

      CATACGTTGT ATCCATATCA TAATATGTAC ATTTATATTG GCTCATGTCC AACATTACCG    6240

      CCATGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT   ·6300

 15   CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA    6360

      CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA    6420

      ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA    6480

      GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG    6540

 20   CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC    6600

      TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT    6660

      GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT    6720

      TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG    6780

      ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TCGTTTAGTG    6840

 25   AACCGTCAGA TCGCCTGGAG ACGCCATCCA CGCTGTTTTG ACCTCCATAG AAGACACCGG    6900
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     GACCGATCCA GCCTCCGCGG CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG   6960
   1 AGTGACGTAA GTACCGCCTA TAGAGTCTAT AGGCCCACCC CCTTGGCTTC TTATGCATGC   7020
     TATACTGTTT TTGGCTTCGG GTCTATACAC CCCCGCTTCC TCATGTTATA GGTGATGGTA   7080
     TAGCTTAGCC TATAGGTGTG GGTTATTGAC CATTATTGAC CACTCCCCTA TTGGTGACGA   7140
   5 TACTTTCCAT TACTAATCCA TAACATGGCT CTTTGCCACA ACTCTCTTTA TTGGCTATAT   7200
     GCCAATACAC TGTCCTTCAG AGACTGACAC GGACTCTGTA TTTTTACAGG ATGGGGTCTC   7260
     ATTTATTATT TACAAATTCA CATATACAAC ACCACCGTCC CCAGTGCCCG CAGTTTTTAT   7320
     TAAACATAAC GTGGGATCTC CACGCGAATC TCGGGTACGT GTTCCGGACA TGGGCTCTTC   7380
     TCCGGTAGCG GCGGAGCTTC TACATCCGAG CCCTGCTCCC ATGCCTCCAG CGACTCATGG   7440
  10 TCGCTCGGCA TCTCCTTGCT CCTAACAGTG GAGGCCAGAC TTAGGCACAG CACGATGCCC   7500
     ACCACCACCA GTGTGCCGCA CAAGGCCGTG GCGGTAGGGT ATGTGTCTGA AAATGAGCTC   7560
     GGGGAGCGGG CTTGCACCGC TGACGCATTT GGAAGACTTA AGGCAGCGGC AGAAGAAGAT   7620
     GCAGGCAGCT GAGTTGTTGT GTTCTGATAA GAGTCAGAGG TAACTCCCGT TGCGGTGCTG   7680
     TTAACGGTGG AGGGCAGTGT AGTCTGAGCA GTACTCGTTG CTGCCGCGCG CGCCACCAGA   7740
  15 CATAATAGCT GACAGACTAA CAGACTGTTC CTTTCCATGG GTCTTTTCTG CAGTCACCGT   7800
     CCTTGACACG AAGCTTGGGC TGCAGGTCGA TCGACTCTAG AGGATCGATC CCCGGGCGAG   7860
     CTCG                                                              7864
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WHAT IS CLAIMED IS:

1.   A CDR-grafted antibody capable of inhibiting human tissue factor wherein the complementarity determining regions (CDRs) are derived from a non-human monoclonal antibody against tissue factor and the framework (FR) and constant (C) regions are derived from one or more human antibodies.

2.   The CDR-grafted antibody of Claim 1 wherein said non-human monoclonal antibody is a murine antibody.

3.   The CDR-grafted antibody of Claim 2 wherein said murine antibody is TF8-5G9.

4.   The CDR-grafted antibody of Claim 1 wherein said CDRs of the heavy chain have the amino acid sequences:

CDR1	DDYMH	(SEQ ID NO:5)
CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
CDR3	DNSYYFDY	(SEQ ID NO:7)

and said CDRs of the light chain have the amino acid sequences:

CDR1	KASQDIRKYLN	(SEQ ID NO:8)
CDR2	YATSLAD	(SEQ ID NO:9)
CDR3	LQHGESPYT	(SEQ ID NO:10).

5.   The CDR-grafted antibody of Claim 1 wherein the FR of the heavy chain is derived from the human antibody KOL.

6.   The CDR-grafted antibody of Claim 1 wherein the FR of the light chain is derived from the human antibody REI.

7.   The CDR-grafted antibody of Claim 1
1 wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:11.

8.   The CDR-grafted antibody of Claim 1 or 7
wherein the light chain variable region has the amino
5 acid sequence of SEQ ID NO:12.

9.   The CDR-grafted antibody of Claim 1
wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:13.

10.   The CDR-grafted antibody of Claim 1 or 9
10 wherein the light chain variable region has the amino
acid sequence of SEQ ID NO:14.

11.   The CDR-grafted antibody of Claim 1
wherein the heavy chain constant region is the human
IgG4 constant region.

15   12.   The CDR-grafted antibody of Claim 10
wherein the heavy chain constant region is the human
IgG4 constant region.

13.   The CDR-grafted antibody of Claim 1
wherein the light chain constant region is the human
20 kappa constant region.

14.   The CDR-grafted antibody of Claim 10
wherein the light chain constant region is the human
kappa constant region.

15.   CDR-grafted monoclonal antibody TF8HCDR1
25 x TF8LCDR1.

16.   CDR-grafted monoclonal antibody TF8HCDR20
x TF8LCDR3.

17.   A fragment of the CDR-grafted antibody of
Claim 1 wherein said fragment is capable of inhibiting
30 human tissue factor.

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18. The fragment of Claim 17 wherein said
1 fragment is an Fab or F(ab')₂ fragment.

19. A method of making the CDR-grafted
antibody of Claim 1 comprising cotransfecting a host
cell with an expression vector comprising a nucleic acid
5 encoding the CDR-grafted antibody heavy chain and an
expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain; culturing the
transfected host cell; and recovering said CDR-grafted
antibody.

10   20. A method of making the CDR-grafted
antibody of Claim 1 comprising transfecting a host cell
with an expression vector comprising a nucleic acid
encoding the CDR-grafted antibody heavy chain and a
nucleic acid encoding the CDR-grafted antibody light
15 chain; culturing the transfected host cell; and
recovering said CDR-grafted antibody.

21. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted antibody heavy
chain has the sequence of nucleotides 1-2360 of SEQ ID
20 NO:15.

22. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted light chain has
the sequence of nucleotides 1-759 of SEQ ID NO:17.

23. The method of Claim 19 or 20 wherein said
25 host cell is a bacterial cell, yeast cell, insect cell
or mammalian cell.

24. The method of Claim 23 wherein said
mammalian cell is a CHO cell, COS cell or myeloma cell.

25. The method of Claim 19 wherein said
30 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody heavy chain is pEe6TF8HCDR20.

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26. The method of Claim 19 wherein said
1 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain is pEe12TF8LCDR3.

27. A nucleic acid encoding the heavy chain
of the CDR-grafted antibody of Claim 1.
5   28. A nucleic acid encoding the light chain
of the CDR-grafted antibody of Claim 1.

29. The nucleic acid of Claim 27 having the
sequence of nucleotides 1-2360 of SEQ ID NO:15.

30. The nucleic acid of Claim 28 having the
10 sequence of nucleotides 1-759 of SEQ ID NO:17.

31. A method of attenuation of coagulation
comprising administering a therapeutically effective
amount of a CDR-grafted antibody capable of inhibiting
human tissue factor to a patient in need of said
15 attenuation.

32. The method of Claim 31 wherein said CDR-
grafted antibody is TF8HCDR20 x TF84CDR3.

33. A method of treatment or prevention of
thrombotic disorder comprising administering a
20 therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
patient in need of said treatment or prevention.

34. The method of Claim 33 wherein said
thrombotic disorder is intravascular coagulation,
25 arterial restenosis or arteriosclerosis.

35. The method of Claim 33 or 34 wherein said
CDR-grafted antibody is TF8HCDR20 x TF8LCDR3.

36. A pharmaceutical composition comprising
at least one CDR-grafted antibody capable of inhibiting
30 human tissue factor and a pharmaceutically acceptable
carrier.

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     37.   The pharmaceutical composition of Claim

1 36 wherein said CDR-grafted antibody is TF8HCDR20 x
   TF8LCDR3.

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Sequence of the murine TF8-5G9 heavy chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 1 A

Nucleotides	Region
1-10	5' untranslated region.
11-67	Start codon and leader sequence.
68-418	Variable region.
419-1390	Murine IgG1 constant region.
1391-1489	3' untranslated region.

Sequence Range: 1 to 1489

```
          10            20            30            40
           •             •             •             •
GGT CCT TAC A ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG
CCA GGA ATG T TAC TTT ACG TCG ACC CAG TAG AAG AAG GAC TAC CGT CAC
          Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val>

 50            60            70            80            90
  •             •             •             •             •
GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG
CAA TGT CCC CAG TTA AGT CTC TAA GTC GAC GTC GTC AGA CCC CGA CTC
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu>

 100           110           120           130           140
  •             •             •             •             •
CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC
GAA CAC TCC GGT CCC CGG AAT CAG TTC AAC AGG ACG TTT CGA AGA CCG
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly>

    150           160           170           180           190
     •             •             •             •             •
TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA
AAG TTG TAA TTT CTG ATG ATA TAC GTG ACC CAC TTC GTC TCC GGA CTT
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu>

    200           210           220           230           240
     •             •             •             •             •
CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT
GTC CCG GAC CTC ACC TAA CCT AAC TAA CTA GGA CTC TTA CCA TTA TGA
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr>

    250           260           270           280
     •             •             •             •
ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA
TAT ATA CTG GGC TTC AAG GTC CCG TTC CGG TCA TAT TGT CGT CTG TGT
Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr>

 290           300           310           320           330
  •             •             •             •             •
TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC
AGG AGG TTG TGT CGG ATG GAC GTC GAG TCG TCG GAC TGT AGA CTC CTG
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp>
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RECTIFIED SHEET (RULE 91)

ISA/EP

FIG. 1 B

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      340           350           360           370           380
       *             *             *             *             *
ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC
TGA CGG CAG ATA ATG ACA CGA TCT CTA TTG AGC ATG ATG AAA CTG ATG
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr>


      390           400           410           420           430
       *             *             *             *             *
TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC
ACC CCG GTT CCG TGG TGA GAG TGT CAG AGG AGT CGG TTT TGC TGT GGG
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro>


      440           450           460           470           480
       *             *             *             *             *
CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC
GGT AGA CAG ATA GGT GAC CGG GGA CCT AGA CGA CGG GTT TGA TTG AGG
Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser>


      490           500           510           520
       *             *             *             *
ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG
TAC CAC TGG GAC CCT ACG GAC CAG TTC CCG ATA AAG GGA CTC GGT CAC
Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val>


  530           540           550           560           570
   *             *             *·             *             *
ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC
TGT CAC TGG ACC TTG AGA CCT AGG GAC AGG TCG CCA CAC GTG TGG AAG
Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe>


  580           590           600           610           620
   *             *             *             *             *
CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT
GGT CGA CAG GAC GTC AGA CTG GAG ATG TGA GAC TCG TCG AGT CAC TGA
Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr>


      630           640           650           660           670
       *             *             *             *             *
GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC
CAC GGG AGG TCG TGG ACC GGG TCG CTC TGG CAG TGG ACG TTG CAA CGG
Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala>


          680           690           700           710           720
           *             *             *             *             *
CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTC CCC AGG GAT
GTG GGC CGG TCG TCG TGG TTC CAC CTG TTC TTT TAA CAC GGG TCC CTA
His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp>


          730           740           750           760
           *             *             *             *
TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC
ACA CCA ACA TTC GGA ACG TAT ACA TGT CAG GGT CTT CAT AGT AGA CAG
Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val>


  770           780           790           800           810
   *             *             *             *             *
TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT
AAG TAG AAG GGG GGT TTC GGG TTC CTA CAC GAG TGG TAA TGA GAC TGA
Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr>
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FIG. 1 C

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      820             830             840             850             860
       •               •               •               •               •
CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG
GGA TTC CAG TGC ACA CAA CAC CAT CTG TAG TCG TTC CTA CTA GGG CTC
Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu>


          870             880             890             900             910
           •               •               •               •               •
GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG
CAG GTC AAG TCG ACC AAA CAT CTA CTA CAC CTC CAC GTG TGT CGA GTC
Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln>


          920             930             940             950             960
           •               •               •               •               •
ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT
TGC GTT GGG GCC CTC CTC GTC AAG TTG TCG TGA AAG GCG AGT CAG TCA
Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser>


          970             980             990             1000
           •               •               •               •
GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA
CTT GAA GGG TAG TAC GTG GTC CTG ACC GAG TTA CCG TTC CTC AAG TTT
Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys>


1010             1020             1030             1040             1050
 •                •                •                •                •
TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC
ACG TCC CAG TTG TCA CGT CGA AAG GGA CGG GGG TAG CTC TTT TGG TAG
Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile>


1060             1070             1080             1090             1100
 •                •                •                •                •
TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA
AGG TTT TGG TTT CCG TCT GGC TTC CGA GGT GTC CAC ATG TGG TAA GGT
Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro>


1110             1120             1130             1140             1150
 •                •                •                •                •
CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG ACC TGC ATG
GGA GGG TTC CTC GTC TAC CGG TTC CTA TTT CAG TCA GAC TGG ACG TAC
Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met>


1160             1170             1180             1190             1200
 •                •                •                •                •
ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT
TAT TGT CTG AAG AAG GGA CTT CTG TAA TGA CAC CTC ACC GTC ACC TTA
Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn>


1210             1220             1230             1240
 •                •                •                •
GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA
CCC GTC GGT CGC CTC TTG ATG TTC TTG TGA GTC GGG TAG TAC CTG TGT
Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr>


1250             1260             1270             1280             1290
 •                •                •                •                •
GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC
CTA CCG AGA ATG AAG CAG ATG TCG TTC GAG TTA CAC GTC TTC TCG TTG
Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn>
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FIG. 1 D

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    1300            1310            1320            1330            1340
     •               •               •               •               •
TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG
ACC CTC CGT CCT TTA TGA AAG TGG ACG AGA CAC AAT GTA CTC CCG GAC
Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu>


     1350            1360            1370            1380            1390
      •               •               •               •               •
CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA TG ATC
GTG TTG GTG GTA TGA CTC TTC TCG GAG AGG GTG AGA GGA CCA TTT AC TAG
His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys>


     1400            1410            1420            1430            1440
      •               •               •               •               •
CCA GTG TCC TTG GAG CCC TCT GGT CCT ACA GGA CTC TGA CAC CTA CCT
GGT CAC AGG AAC CTC GGG AGA CCA GGA TGT CCT GAG ACT GTG GAT GGA


     1450            1460            1470             1480
      •               •               •                •
CCA CCC CTC CCT GTA TAA ATA AAG CAC CCA GCA CTG CCT TGG ACC C
GGT GGG GAG GGA CAT ATT TAT TTC GTG GGT CGT GAC GGA ACC TGG G
```

Sequence of the murine TF8-5G9 light chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 2 A

Nucleotides	Region
1-4	5' untranslated.
5-64	Start codon and leader sequence.
65-385	Variable region.
386-706	Murine kappa constant region.
707-917	3' untranslated region.
918-937	Poly A tail.

Sequence Range: 1 to 937

```
          10              20              30              40
           *               *               *               *
GGA C ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT
CCT G TAC GCC CGG GGA CGA GTC AAA AAA CCC TAG AAC AAC GAG ACC AAA
    Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe>

  50          60          70          80          90
   *           *           *           *           *
CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG
GGT CCA TAG TCT ACA CTG TAG TTC TAC TGG GTC AGA GGT AGG AGG TAC
Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met>

  100         110         120         130         140
   *           *           *           *           *
TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG
ATA CGT AGC GAC CCT CTC TCT CAG TGA TAG TGA ACA TTC CGC TCA GTC
Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln>

   150         160         170         180         190
    *           *           *           *           *
GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT
CTG TAA TCT TTC ATA AAT TTG ACC ATG GTC GTC TTT GGT ACC TTT AGA
Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser>

    200         210         220         230         240
     *           *           *           *           *
CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA
GGA TTC TGG GAC TAG ATA ATA CGT TGT TCG AAC CGT CTA CCC CAG GGT
Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro>

     250         260         270         280
      *           *           *           *
TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC
AGT TCT AAG TCA CCG TCA CCT AGA CCC GTT CTA ATA AGA GAT TGG TAG
Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile>

 290         300         310         320         330
  *           *           *           *           *
AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT
TCG TCG GAC CTC AGA CTG CTA TGT CGT TGA ATA ATG ACA GAT GTT GTA
Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His>
```

FIG. 2B

```
     340          350          360          370          380
      • .           •            • ·          •            •
GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC
CCA CTC TCG GGC ATG TGC AAG CCT CCC CCC TGG TTC GAC CTT TAT TTG
Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn>

     390          400          410          420          430
      •            •            •            •            •
AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG
TCC CGA CTA CGA CGT GGT TGA CAT AGG TAG AAG GGT GGT AGG TCA CTC
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu>

     440          450          460          470          480
      •            •            •            •            •
CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC
GTC AAT TGT AGA CCT CCA CGG AGT CAG CAC ACG AAG AAC TTG TTG AAG
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe>

     490          500          510          520
      •            •            •            •
TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA
ATG GGG TTT CTG TAG TTA CAG TTC ACC TTC TAA CTA CCG TCA CTT GCT
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg>

  530          540          550          560          570
   •            •            •            •            •
CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC
GTT TTA CCG CAG GAC TTG TCA ACC TGA CTA GTC CTG TCG TTT CTG TCG
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser>

  580          590          600          610          620
   •            •            •            •            •
ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA
TGG ATG TCG TAC TCG TCG TGG GAG TGC AAC TGG TTC CTC CTC ATA CTT
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu>

  630          640          650          660          670
   •            •            •            •            •
CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA
GCT GTA TTG TCG ATA TGG ACA CTC CGG TGA GTG TTC TGT AGT TGA AGT
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser>

     680          690          700          710          720
      •            •            •            •            •
CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT TA GAG ACA AAG GTC CTG
GGG TAA CAG TTC TCG AAG TTG TCC TTA CTC ACA AT CTC TGT TTC CAG GAC
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys>

     730          740          750          760          770
      •            •            •            •            •
AGA CGC CAC CAC CAG CTC CCC AGC TCC ATC CTA TCT TCC CTT CTA AGG
TCT GCG GTG GTG GTC GAG GGG TCG AGG TAG GAT AGA AGG GAA GAT TCC

        780          790          800          810
         •            •            •            •
TCT TGG AGG CTT CCC CAC AAG CGA CCT ACC ACT GTT GCG GTG CTC CAA
AGA ACC TCC GAA GGG GTG TTC GCT GGA TGG TGA CAA CGC CAC GAG GTT
```

FIG. 2 C

```
    820             830           840          850          860
     *               *             *            *            *
ACC TCC TCC CCA CCT CCT TCT CCT CCT CCT CCC TTT CCT TGG CTT TTA
TGG AGG AGG GGT GGA GGA AGA GGA GGA GGA GGG AAA GGA ACC GAA AAT


    870             880           890          900          910
     *               *             *            *            *
TCA TGC TAA TAT TTG CAG AAA ATA TTC AAT AAA GTG AGT CTT TGC ACT
AGT ACG ATT ATA AAC GTC TTT TAT AAG TTA TTT CAC TCA GAA ACG TGA


      920             930
       *               *
TGA AAA AAA AAA AAA AAA AAA A
ACT TTT TTT TTT TTT TTT TTT T
```

FIG. 3

anti-TF BINDING AND COMPETITION ASSAYS
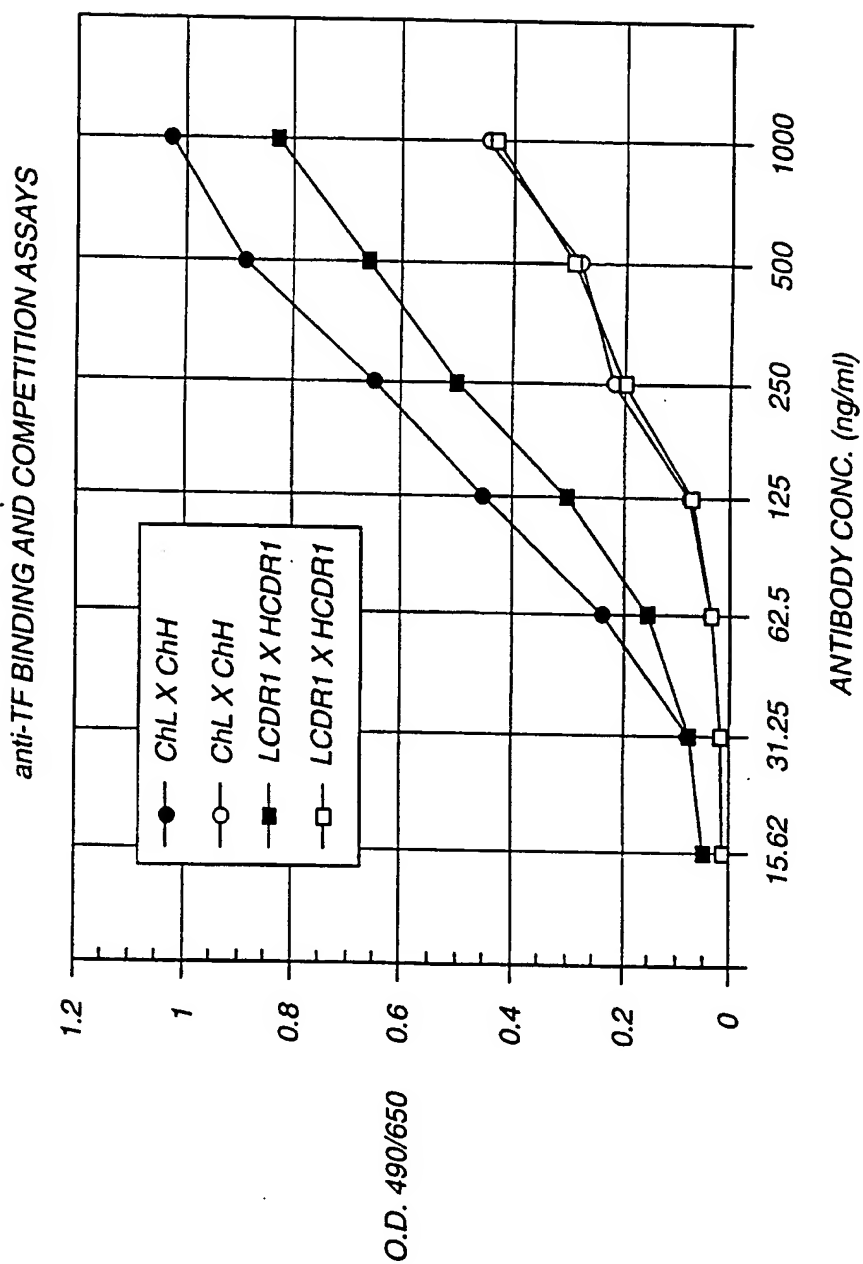
FIG. 4 A

The pEe6TF8HCDR20 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted HC gene, TF8HCDR20, are translated.

Sequence Range: 1 to 7073

```
              10            20            30            40
               •             •             •             •
     GAA TTC GCC GCC ACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC TTG
     CTT AAG CGG CGG TGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAG AAC
                     Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu>

      50            60            70            80            90
       •             •             •             •             •
     TCA GTA ACT ACA GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA
     AGT CAT TGA TGT CCA CAT GTG AGT GTT CAA GTC GAC CAC CTC AGA CCT
     Ser Val Thr Thr Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly>

        100           110           120           130           140
         •             •             •             •             •
     GGA GGA GTA GTA CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT
     CCT CCT CAT CAT GTT GGA CCT TCC AGT GAC TCT GAC AGA ACA TTC CGA
     Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala>

        150           160           170           180           190
         •             •             •             •             •
     AGT GGA TTC AAT ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT
     TCA CCT AAG TTA TAG TTC CTG ATA ATA TAC GTG ACC CAG TCT GTT CGA
     Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala>

          200           210           220           230           240
           •             •             •             •             •
     CCT GGA AAA GGA CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT
     GGA CCT TTT CCT GAG CTC ACC TAT CCA AAT TAA CTA GGA CTC TTA CCA
     Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly>

            250           260           270           280
             •             •             •             •
     AAC ACG ATA TAT GAT CCC AAG TTC CAA GGA AGA TTC ACA ATT TCT GCA
     TTG TGC TAT ATA CTA GGG TTC AAG GTT CCT TCT AAG TGT TAA AGA CGT
     Asn Thr Ile Tyr Asp Pro Lys Phe Gln Gly Arg Phe Thr Ile Ser Ala>

     290           300           310           320           330
      •             •             •             •             •
     GAC AAC TCT AAG AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT
     CTG TTG AGA TTC TTA TGT GAC AAG GAC GTC TAC CTG AGT GAG TCT GGA
     Asp Asn Ser Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro>

       340           350           360           370           380
        •             •             •             •             •
     GAG GAT ACA GCA GTC TAC TAT TGT GCT AGA GAT AAC AGT TAT TAC TTC
     CTC CTA TGT CGT CAG ATG ATA ACA CGA TCT CTA TTG TCA ATA ATG AAG
     Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe>

         390           400           410           420           430
          •             •             •             •             •
     GAC TAC TGG GGC CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC
     CTG ATG ACC CCG GTT CCT TGT GGT CAG TGG CAC TCG AGT CGA AGG TGG
     Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr>
```

FIG. 4 B

```
        440           450           460           470           480
         *            *             *             *             *
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC
TTC CCG GGT AGG CAG AAG GGG GAC CGC GGG ACG AGG TCC TCG TGG AGG
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser>


              490           500           510           520
               *             *             *             *
GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
CTC TCG TGT CGG CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG CTT
Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>


  530           540           550           560           570
   *             *             *             *             *
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>


        580           590           600           610           620
         *             *             *             *             *
ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC
TGG AAG GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>


        630           640           650           660           670
         *             *             *             *             *
GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
CAC CAC TGG CAC GGG AGG TCG TCG AAC CCG TGC TTC TGG ATG TGG ACG
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys>


        680           690           700           710           720
         *             *             *             *             *
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGT
TTG CAT CTA GTG TTC GGG TCG TTG TGG TTC CAC CTG TTC TCT CAA CCA
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val>


              730           740           750           760
               *             *             *             *
GAG AGG CCA GCA CAG GGC AGG GAG GGT GTC TGC TGG AAG CCA GGC TCA
CTC TCC GGT CGT GTC CCG TCC CTC CCA CAG ACG ACC TTC GGT CCG AGT


  770           780           790           800           810
   *             *             *             *             *
GCC CTC CTG CCT GGA CGC ACC CCG GCT GTG CAG CCC CAG CCC AGG GCA
CGG GAG GAC GGA CCT GCG TGG GGC CGA CAC GTC GGG GTC GGG TCC CGT


     820           830           840           850           860
      *             *             *             *             *
GCA AGG CAT GCC CCA TCT GTC TCC TCA CCC GGA GGC CTC TGA CCA CCC
CGT TCC GTA CGG GGT AGA CAG AGG AGT GGG CCT CCG GAG ACT GGT GGG


     870           880           890           900           910
      *             *             *             *             *
CAC TCA TGC TCA GGG AGA GGG TCT TCT GGA TTT TTC CAC CAG GCT CCG
GTG AGT ACG AGT CCC TCT CCC AGA AGA CCT AAA AAG GTG GTC CGA GGC
```

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FIG. 4 C

```
         920         930         940         950         960
          •           •           •           •           •
GGC AGC CAC AGG CTG GAT GCC CCT ACC CCA GGC CCT GCG CAT ACA GGG
CCG TCG GTG TCC GAC CTA CGG GGA TGG GGT CCG GGA CGC GTA TGT CCC


              970         980         990        1000
               •           •           •           •
GCA GGT GCT GCG CTC AGA CCT GCC AAG AGC CAT ATC CGG GAG GAC CCT
CGT CCA CGA CGC GAG TCT GGA CGG TTC TCG GTA TAG GCC CTC CTG GGA


  1010        1020        1030        1040        1050
   •           •           •           •           •
GCC CCT GAC CTA AGC CCA CCC CAA AGG CCA AAC TCT CCA CTC CCT CAG
CGG GGA CTG GAT TCG GGT GGG GTT TCC GGT TTG AGA GGT GAG GGA GTC


     1060        1070        1080        1090        1100
      •           •           •           •           •
CTC AGA CAC CTT CTC TCC TCC CAG ATT CGA GTA ACT CCC AAT CTT CTC
GAG TCT GTG GAA GAG AGG AGG GTC TAA GCT CAT TGA GGG TTA GAA GAG


     1110        1120        1130        1140        1150
      •           •           •           •           •
TCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA GGT AAG
AGA CGT CTC AGG TTT ATA CCA GGG GGT ACG GGT AGT ACG GGT CCA TTC
    Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro>


        1160        1170        1180        1190        1200
         •           •           •           •           •
CCA ACC CAG GCC TCG CCC TCC AGC TCA AGG CGG GAC AGG TGC CCT AGA
GGT TGG GTC CGG AGC GGG AGG TCG AGT TCC GCC CTG TCC ACG GGA TCT


           1210        1220        1230        1240
            •           •           •           •
GTA GCC TGC ATC CAG GGA CAG GCC CCA GCC GGG TGC TGA CGC ATC CAC
CAT CGG ACG TAG GTC CCT GTC CGG GGT CGG CCC ACG ACT GCG TAG GTG


  1250        1260        1270        1280        1290
   •           •           •           •           •
CTC CAT CTC TTC CTC AGC A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC
GAG GTA GAG AAG GAG TCG T GGA CTC AAG GAC CCC CCT GGT AGT CAG AAG
                         Pro Glu Phe Leu Gly Gly Pro Ser Val Phe>


  1300        1310        1320        1330        1340
   •           •           •           •           •
CTG TTC CCC CCA AAA CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT
GAC AAG GGG GGT TTT GGG TTC CTG TGA GAG TAC TAG AGG GCC TGG GGA
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>


     1350        1360        1370        1380        1390
      •           •           •           •           •
GAG GTC ACG TGC GTG GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC
CTC CAG TGC ACG CAC CAC CAC CTG CAC TCG GTC CTT CTG GGG CTC CAG
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val>


     1400        1410        1420        1430        1440
      •           •           •           •           •
CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA
GTC AAG TTG ACC ATG CAC CTA CCG CAC CTC CAC GTA TTA CGG TTC TGT
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>
```

FIG. 4 D

```
              1450             1460             1470             1480
               •                •                •                •
    AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC
    TTC GGC GCC CTC CTC GTC AAG TTG TCG TGC ATG GCA CAC CAG TCG CAG
    Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val>

         1490        1500             1510             1520        1530
          •           •                •                •           •
    CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC
    GAG TGG CAG GAC GTG GTC CTG ACC GAC TTG CCG TTC CTC ATG TTC ACG
    Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

         1540             1550             1560             1570        1580
          •                •                •                •           •
    AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC
    TTC CAG AGG TTG TTT CCG GAG GGC AGG AGG TAG CTC TTT TGG TAG AGG
    Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser>

          1590             1600             1610             1620        1630
           •                •                •                •           •
    AAA GCC AAA GG TGG GAC CCA CGG GGT GCG AGG GCC ACA TGG ACA GAG GTC
    TTT CGG TTT CC ACC CTG GGT GCC CCA CGC TCC CGG TGT ACC TGT CTC CAG
    Lys Ala Lys>

         1640             1650             1660             1670        1680
          •                •                •                •           •
    AGC TCG GCC CAC CCT CTG CCC TGG GAG TGA CCG CTG TGC CAA CCT CTG
    TCG AGC CGG GTG GGA GAC GGG ACC CTC ACT GGC GAC ACG GTT GGA GAC

              1690        1700             1710             1720        1730
               •           •                •                •           •
        TCC CTA CA GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC
        AGG GAT GT CCC GTC GGG GCT CTC GGT GTC CAC ATG TGG GAC GGG GGT AGG
                  Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser>

              1740        1750             1760             1770        1780
               •           •                •                •           •
    CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA
    GTC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
    Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

              1790             1800             1810             1820
               •                •                •                •
        GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
        CCG AAG ATG GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC
        Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>

    1830        1840             1850             1860             1870
     •           •                •                •                •
    CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
    GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG
    Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>

         1880        1890             1900             1910        1920
          •           •                •                •           •
    TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG
    AGG AAG AAG GAG ATG TCG TCC GAT TGG CAC CTG TTC TCG TCC ACC GTC
    Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln>
```

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FIG. 4 E

```
        1930        1940        1950        1960        1970
         •           •           •           •           •
GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC
CTC CCC TTA CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>

        1980        1990        2000        2010        2020
         •           •           •           •           •
CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA T GAG TGC CAG
GTG ATG TGT GTC TTC TCG GAG AGG GAC AGA GAC CCA TTT A CTC ACG GTC
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Xxx>

        2030        2040        2050        2060        2070
         •           •           •           •           •
GGC CGG CAA GCC CCC GCT CCC CGG GCT CTC GGG GTC GCG CGA GGA TGC
CCG GCC GTT CGG GGG CGA GGG GCC CGA GAG CCC CAG CGC GCT CCT ACG

        2080        2090        2100        2110
         •           •           •           •
TTG GCA CGT ACC CCG TCT ACA TAC TTC CCA GGC ACC CAG CAT GGA AAT
AAC CGT GCA TGG GGC AGA TGT ATG AAG GGT CCG TGG GTC GTA CCT TTA

2120        2130        2140        2150        2160
  •           •           •           •           •
AAA GCA CCC ACC ACT GCC CTG GGC CCC TGT GAG ACT GTG ATG GTT CTT
TTT CGT GGG TGG TGA CGG GAC CCG GGG ACA CTC TGA CAC TAC CAA GAA

        2170        2180        2190        2200        2210
         •           •           •           •           •
TCC ACG GGT CAG GCC GAG TCT GAG GCC TGA GTG ACA TGA GGG AGG CAG
AGG TGC CCA GTC CGG CTC AGA CTC CGG ACT CAC TGT ACT CCC TCC GTC

        2220        2230         2240        2250        2260
         •           •           •           •           •
AGC GGG TCC CAC TGT CCC CAC ACT GGC CCA GGC TGT GCA GGT GTG CCT
TCG CCC AGG GTG ACA GGG GTG TGA CCG GGT CCG ACA CGT CCA CAC GGA

        2270        2280        2290        2300        2310
         •           •           •           •           •
GGG CCA CCT AGG GTG GGG CTC AGC CAG GGG CTG CCC TCG GCA GGG TGG
CCC GGT GGA TCC CAC CCC GAG TCG GTC CCC GAC GGG AGC CGT CCC ACC

        2320        2330        2340        2350
         •           •           •           •
GGG ATT TGC CAG CGT GGC CCT CCC TCC AGC AGC AGG ACT CTA GAG GAT
CCC TAA ACG GTC GCA CCG GGA GGG AGG TCG TCG TCC TGA GAT CTC CTA

2360        2370        2380        2390        2400
  •           •           •           •           •
CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT TGC TTT AAA AAA
GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA ACG AAA TTT TTT

        2410        2420        2430        2440        2450
         •           •           •           •           •
CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT GAA TGC AAT TGT
GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA CTT ACG TTA ACA
```

FIG. 4 F

```
        2460            2470            2480            2490            2500
         •               •               •               •               •
TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA CAA ATA AAG CAA
ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT GTT TAT TTC GTT

        2510            2520            2530            2540            2550
         •               •               •               •               •
TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC ACT GCA TTC TAG
ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG TGA CGT AAG ATC

        2560            2570            2580            2590
         •               •               •               •
TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA TGT CTG GAT CCT
AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT ACA GAC CTA GGA

  2600          2610            2620            2630            2640
   •             •               •               •               •
CTA CGC CGG ACG CAT CGT GGC CGG CAT CAC CGG CGC CAC AGG TGC GGT
GAT GCG GCC TGC GTA GCA CCG GCC GTA GTG GCC GCG GTG TCC ACG CCA

      2650            2660            2670            2680            2690
       •               •               •               •               •
TGC TGG CGC CTA TAT CGC CGA CAT CAC CGA TGG GGA AGA TCG GGC TCG
ACG ACC GCG GAT ATA GCG GCT GTA GTG GCT ACC CCT TCT AGC CCG AGC

      2700            2710            2720            2730            2740
       •               •               •               •               •
CCA CTT CGG GCT CAT GAG CGC TTG TTT CGG CGT GGG TAT GGT GGC AGG
GGT GAA GCC CGA GTA CTC GCG AAC AAA GCC GCA CCC ATA CCA CCG TCC

          2750            2760            2770            2780            2790
           •               •               •               •               •
CCC GTG GCC GGG GGA CTG TTG GGC GCC ATC TCC TTG CAT GCA CCA TTC
GGG CAC CGG CCC CCT GAC AAC CCG CGG TAG AGG AAC GTA CGT GGT AAG

          2800            2810            2820            2830
           •               •               •               •
CTT GCG GCG GCG GTG CTC AAC GGC CTC AAC CTA CTA CTG GGC TGC TTC
GAA CGC CGC CGC CAC GAG TTG CCG GAG TTG GAT GAT GAC CCG ACG AAG

  2840          2850            2860            2870            2880
   •             •               •               •               •
CTA ATG CAG GAG TCG CAT AAG GGA GAG CGT CGA CCT CGG GCC GCG TTG
GAT TAC GTC CTC AGC GTA TTC CCT CTC GCA GCT GGA GCC CGG CGC AAC

      2890            2900            2910            2920            2930
       •               •               •               •               •
CTG GCG TTT TTC CAT AGG CTC CGC CCC CCT GAC GAG CAT CAC AAA AAT
GAC CGC AAA AAG GTA TCC GAG GCG GGG GGA CTG CTC GTA GTG TTT TTA

      2940            2950            2960            2970            2980
       •               •               •               •               •
CGA CGC TCA AGT CAG AGG TGG CGA AAC CCG ACA GGA CTA TAA AGA TAC
GCT GCG AGT TCA GTC TCC ACC GCT TTG GGC TGT CCT GAT ATT TCT ATG
```

FIG. 4 G

```
        2990           3000           3010           3020           3030
         •             •             •             •             •
CAG GCG TTT CCC CCT GGA AGC TCC CTC GTG CGC TCT CCT GTT CCG ACC
GTC CGC AAA GGG GGA CCT TCG AGG GAG CAC GCG AGA GGA CAA GGC TGG


              3040           3050           3060           3070
               •             •             •             •
CTG CCG CTT ACC GGA TAC CTG TCC GCC TTT CTC CCT TCG GGA AGC GTG
GAC GGC GAA TGG CCT ATG GAC AGG CGG AAA GAG GGA AGC CCT TCG CAC


   3080           3090           3100           3110           3120
    •             •             •             •             •
GCG CTT TCT CAA TGC TCA CGC TGT AGG TAT CTC AGT TCG GTG TAG GTC
CGC GAA AGA GTT ACG AGT GCG ACA TCC ATA GAG TCA AGC CAC ATC CAG


      3130           3140           3150           3160           3170
       •             •             •             •             •
GTT CGC TCC AAG CTG GGC TGT GTG CAC GAA CCC CCC GTT CAG CCC GAC
CAA GCG AGG TTC GAC CCG ACA CAC GTG CTT GGG GGG CAA GTC GGG CTG


         3180           3190           3200           3210           3220
          •             •             •             •             •
CGC TGC GCC TTA TCC GGT AAC TAT CGT CTT GAG TCC AAC CCG GTA AGA
GCG ACG CGG AAT AGG CCA TTG ATA GCA GAA CTC AGG TTG GGC CAT TCT


            3230           3240           3250           3260           3270
             •             •             •             •             •
CAC GAC TTA TCG CCA CTG GCA GCA GCC ACT GGT AAC AGG ATT AGC AGA
GTG CTG AAT AGC GGT GAC CGT CGT CGG TGA CCA TTG TCC TAA TCG TCT


              3280           3290           3300           3310
               •             •             •             •
GCG AGG TAT GTA GGC GGT GCT ACA GAG TTC TTG AAG TGG TGG CCT AAC
CGC TCC ATA CAT CCG CCA CGA TGT CTC AAG AAC TTC ACC ACC GGA TTG


   3320           3330           3340           3350           3360
    •             •             •             •             •
TAC GGC TAC ACT AGA AGG ACA GTA TTT GGT ATC TGC GCT CTG CTG AAG
ATG CCG ATG TGA TCT TCC TGT CAT AAA CCA TAG ACG CGA GAC GAC TTC


      3370           3380           3390           3400           3410
       •             •             •             •             •
CCA GTT ACC TTC GGA AAA AGA GTT GGT AGC TCT TGA TCC GGC AAA CAA
GGT CAA TGG AAG CCT TTT TCT CAA CCA TCG AGA ACT AGG CCG TTT GTT


      3420           3430           3440           3450           3460
       •             •             •             •             •
ACC ACC GCT GGT AGC GGT GGT TTT TTT GTT TGC AAG CAG CAG ATT ACG
TGG TGG CGA CCA TCG CCA CCA AAA AAA CAA ACG TTC GTC GTC TAA TGC


         3470           3480           3490           3500           3510
          •             •             •             •             •
CGC AGA AAA AAA GGA TCT CAA GAA GAT CCT TTG ATC TTT TCT ACG GGG
GCG TCT TTT TTT CCT AGA GTT CTT CTA GGA AAC TAG AAA AGA TGC CCC
```

FIG. 4 H

```
        3520           3530           3540           3550
         •             •  •            •              •
TCT GAC GCT CAG TGG AAC GAA AAC TCA CGT TAA GGG ATT TTG GTC ATG
AGA CTG CGA GTC ACC TTC CTT TTG AGT GCA ATT CCC TAA AAC CAG TAC

3560        3570           3580           3590           3600
 •          •              •              •              •
AGA TTA TCA AAA AGG ATC TTC ACC TAG ATC CTT TTA AAT TAA AAA TGA
TCT AAT AGT TTT TCC TAG AAG TGG ATC TAG GAA AAT TTA ATT TTT ACT

    3610        3620           3630           3640           3650
     •          •              •              •              •
AGT TTT AAA TCA ATC TAA AGT ATA TAT GAG TAA ACT TGG TCT GAC AGT
TCA AAA TTT AGT TAG ATT TCA TAT ATA CTC ATT TGA ACC AGA CTG TCA

       3660           3670           3680           3690           3700
        •              •              •              •              •
TAC CAA TGC TTA ATC AGT GAG GCA CCT ATC TCA GCG ATC TGT CTA TTT
ATG GTT ACG AAT TAG TCA CTC CGT GGA TAG AGT CGC TAG ACA GAT AAA

           3710           3720           3730           3740           3750
            •              •              •              •              •
CGT TCA TCC ATA GTT GCC TGA CTC CCC GTC GTG TAG ATA ACT ACG ATA
GCA AGT AGG TAT CAA CGG ACT GAG GGG CAG CAC ATC TAT TGA TGC TAT

              3760           3770           3780           3790
               •              •              •              •
CGG GAG GGC TTA CCA TCT GGC CCC AGT GCT GCA ATG ATA CCG CGA GAC
GCC CTC CCG AAT GGT AGA CCG GGG TCA CGA CGT TAC TAT GGC GCT CTG

3800        3810           3820           3830           3840
 •          •              •              •              •
CCA CGC TCA CCG GCT CCA GAT TTA TCA GCA ATA AAC CAG CCA GCC GGA
GGT GCG AGT GGC CGA GGT CTA AAT AGT CGT TAT TTG GTC GGT CGG CCT

   3850        3860           3870           3880           3890
    •          •              •              •              •
AGG GCC GAG CGC AGA AGT GGT CCT GCA ACT TTA TCC GCC TCC ATC CAG
TCC CGG CTC GCG TCT TCA CCA GGA CGT TGA AAT AGG CGG AGG TAG GTC

   3900           3910           3920           3930           3940
    •              •              •              •              •
TCT ATT AAT TGT TGC CGG GAA GCT AGA GTA AGT AGT TCG CCA GTT AAT
AGA TAA TTA ACA ACG GCC CTT CGA TCT CAT TCA TCA AGC GGT CAA TTA

      3950           3960           3970           3980           3990
       •              •              •              •              •
AGT TTG CGC AAC GTT GTT GCC ATT GCT ACA GGC ATC GTG GTG TCA CGC
TCA AAC GCG TTG CAA CAA CGG TAA CGA TGT CCG TAG CAC CAC AGT GCG

         4000           4010           4020           4030
          •              •              •              •
TCG TCG TTT GGT ATG GCT TCA TTC AGC TCC GGT TCC CAA CGA TCA AGG
AGC AGC AAA CCA TAC CGA AGT AAG TCG AGG CCA AGG GTT GCT AGT TCC
```

FIG. 4 I

```
      4040          4050          4060          4070          4080
       •             •             •             •             •
    CGA GTT ACA TGA TCC CCC ATG TTC TGC AAA AAA GCG GTT AGC TCC TTC
    GCT CAA TGT ACT AGG GGG TAC AAC ACG TTT TTT CGC CAA TCG AGG AAG

          4090          4100          4110          4120          4130
           •             •             •             •             •
    GGT CCT CCC ATC GTT GTC AGA AGT AAG TTG GCC GCA GTG TTA TCA CTC
    CCA GGA GGC TAG CAA CAG TCT TCA TTC AAC CGG CGT CAC AAT AGT GAG

            4140          4150          4160          4170          4180
             •             •             •             •             •
    ATG GTT ATG GCA GCA CTG CAT AAT TCT CTT ACT GTC ATG CCA TCC GTA
    TAC CAA TAC CGT CGT GAC GTA TTA AGA GAA TGA CAG TAC GGT AGG CAT

              4190          4200          4210          4220          4230
               •             •             •             •             •
    AGA TGC TTT TCT GTG ACT GGT GAG TAC TCA ACC AAG TCA TTC TGA GAA
    TCT ACG AAA AGA CAC TGA CCA CTC ATG AGT TGG TTC AGT AAG ACT CTT

                4240          4250          4260          4270
                 •             •             •             •
    TAG TGT ATC CGG CGA CCG AGT TGC TCT TGC CCG GCG TCA ACA CGG GAT
    ATC ACA TAC GCC GCT GGC TCA ACG AGA ACG GGC CGC AGT TGT GCC CTA

      4280          4290·         4300          4310          4320
       •             •             •             •             •
    AAT ACC GCG CCA CAT AGC AGA ACT TTA AAA GTG CTC ATC ATT GGA AAA
    TTA TGG CGC GGT GTA TCG TCT TGA AAT TTT CAC GAG TAG TAA CCT TTT

          4330          4340          4350          4360          4370
           •             •             •             •             •
    CGT TCT TCG GGG CGA AAA CTC TCA AGG ATC TTA CCG CTG TTG AGA TCC
    GCA AGA AGC CCC GCT TTT GAG AGT TCC TAG AAT GGC GAC AAC TCT AGG

            4380          4390          4400          4410          4420
             •             •             •             •             •
    AGT TCG ATG TAA CCC ACT CGT GCA CCC AAC TGA TCT TCA GCA TCT TTT
    TCA AGC TAC ATT GGG TGA GCA CGT GGG TTG ACT AGA AGT CGT AGA AAA

              4430          4440          4450          4460          4470
               •             •             •             •             •
    ACT TTC ACC AGC GTT TCT GGG TGA GCA AAA ACA GGA AGG CAA AAT GCC
    TGA AAG TGG TCG CAA AGA CCC ACT CGT TTT TGT CCT TCC GTT TTA CGG

                4480          4490          4500          4510
                 •             •             •             •
    GCA AAA AAG GGA ATA AGG GCG ACA CGG AAA TGT TGA ATA CTC ATA CTC
    CGT TTT TTC CCT TAT TCC CGC TGT GCC TTT ACA ACT TAT GAG TAT GAG

      4520          4530          4540          4550          4560
       •             •             •             •             •
    TTC CTT TTT CAA TAT TAT TGA AGC ATT TAT CAG GGT TAT TGT CTC ATG
    AAG GAA AAA GTT ATA ATA ACT TCG TAA ATA GTC CCA ATA ACA GAG TAC
```

FIG. 4 J

```
      4570          4580          4590          4600          4610
       •            •            •              •            •
AGC GGA TAC ATA TTT GAA TGT ATT TAG AAA AAT AAA CAA ATA GGG GTT
TCG CCT ATG TAT AAA CTT ACA TAA ATC TTT TTA TTT GTT TAT CCC CAA

      4620          4630          4640          4650          4660
       •            •            •            •            •
CCG CGC ACA TTT CCC CGA AAA GTG CCA CCT GAC GTC TAA GAA ACC ATT
GGC GCG TGT AAA GGG GCT TTT CAC GGT CGA CTC CAG ATT CTT TGG TAA

      4670          4680          4690          4700          4710
       •            •            •            •            •
ATT ATC ATG ACA TTA ACC TAT AAA AAT AGG CGT ATC ACG AGG CCC TGA
TAA TAG TAC TGT AAT TGG ATA TTT TTA TCC GCA TAG TGC TCC GGG ACT

         4720          4730          4740          4750
          •            •            •            •
TGG CTC TTT GCG GCA CCC ATC GTT CGT AAT GTT CCG TGG CAC CGA GGA
ACC GAG AAA CGC CGT GGG TAG CAA GCA TTA CAA GGC ACC GTG GCT CCT

 4760          4770          4780          4790          4800
  •            •            •            •            •
CAA CCC TCA AGA GAA AAT GTA ATC ACA CTG GCT CAC CTT CCG GTG GGC
GTT GGG AGT TCT CTT TTA CAT TAG TGT GAC CGA GTG GAA GCC CAC CCG

      4810          4820          4830          4840          4850
       •            •            •            •            •
CTT TCT GCG TTT ATA AGG AGA CAC TTT ATG TTT AAG AAG GTT GGT AAA
GAA AGA CGC AAA TAT TCC TCT GTG AAA TAC AAA TTC TTC CAA CCA TTT

       4860          4870          4880          4890          4900
        •            •            •            •            •
TTC CTT GCG GCT TTG GCA GCC AAG CTA GAG ATC TCT AGC TTC GTG TCA
AAG GAA CGC CGA AAC CGT CGG TTC GAT CTC TAG AGA TCG AAG CAC AGT

      4910          4920          4930          4940          4950
       •            •            •            •            •
AGG ACG GTG ACT GCA GTC AAT AAT AAA ATG TGT GTT TGT CCG AAA TAC
TCC TGC CAC TGA CGT CAC TTA TTA TTT TAC ACA CAA ACA GGC TTT ATG

       4960          4970          4980          4990
        •            •            •            •
GCG TTT TGA GAT TTC TGT CGC CGA CTA AAT TCA TGT CGC CGG ATA GTG
CGC AAA ACT CTA AAG ACA GCG GCT GAT TTA AGT ACA GCG CGC TAT CAC

 5000          5010          5020          5030          5040
  •            •            •            •            •
GTG TTT ATC GCC GAT AGA GAT GGC GAT ATT GGA AAA ATC GAT ATT TGA
CAC AAA TAG CGG CTA TCT CTA CCG CTA TAA CCT TTT TAG CTA TAA ACT

      5050          5060          5070          5080          5090
       •            •            •            •            •
AAA TAT GGC ATA TTG AAA ATG TCG CCG ATG TGA GTT TCT GTG TAA CTG
TTT ATA CCG TAT AAC TTT TAC AGC GGC TAC ACT CAA AGA CAC ATT GAC
```

FIG. 4 K

```
        5100          5110          5120          5130          5140
          •            •            •            •            •
ATA TCG CCA TTT TTC CAA AAG TGA TTT TTG GGC ATA CGC GAT ATC TGG
TAT AGC GGT AAA AAG GTT TTC ACT AAA AAC CCG TAT GCG CTA TAG ACC

          5150          5160          5170          5180          5190
            •            •            •            •            •
CGA TAG CGC TTA TAT CGT TTA CGG GGG ATG GCG ATA GAC GAC TTT GGT
GCT ATC GCG AAT ATA GCA AAT GCC CCC TAC CGC TAT CTG CTG AAA CCA

          5200          5210          5220          5230
            •            •            •            •
GAC TTG GGC GAT TCT GTG TGT CGC AAA TAT CGC AGT TTC GAT ATA GGT
CTG AAC CCG CTA AGA CAC ACA GCG TTT ATA GCG TCA AAG CTA TAT CCA

     5240          5250          5260          5270          5280
       •            •            •            •            •
GAC AGA CGA TAT GAG GCT ATA TCG CCG ATA GAG GCG ACA TCA AGC TGG
CTG TCT GCT ATA CTC CGA TAT AGC GGC TAT CTC CGC TGT AGT TCG ACC

        5290          5300          5310          5320          5330
          •            •            •            •            •
CAC ATG GCC AAT GCA TAT CGA TCT ATA CAT TGA ATC AAT ATT GGC CAT
GTG TAC CGG TTA CGT ATA GCT AGA TAT GTA ACT TAG TTA TAA CCG GTA

        5340          5350          5360          5370          5380
          •            •            •            •            •
TAG CCA TAT TAT TCA TTG GTT ATA TAG CAT AAA TCA ATA TTG GCT ATT
ATC GGT ATA ATA AGT AAC CAA TAT ATC GTA TTT AGT TAT AAC CGA TAA

          5390          5400          5410          5420          5430
            •            •            •            •            •
GGC CAT TGC ATA CGT TGT ATC CAT ATC ATA ATA TGT ACA TTT ATA TTG
CCG GTA ACG TAT GCA ACA TAG GTA TAG TAT TAT ACA TGT AAA TAT AAC

          5440          5450          5460          5470
            •            •            •            •
GCT CAT GTC CAA CAT TAC CGC CAT GTT GAC ATT GAT TAT TGA CTA GTT
CGA GTA CAG GTT GTA ATG GCG GTA CAA CTG TAA CTA ATA ACT GAT CAA

     5480          5490          5500          5510          5520
       •            •            •            •            •
ATT AAT AGT AAT CAA TTA CGG GGT CAT TAG TTC ATA GCC CAT ATA TGG
TAA TTA TCA TTA GTT AAT GCC CCA GTA ATC AAG TAT CGG GTA TAT ACC

        5530          5540          5550          5560          5570
          •            •            •            •            •
AGT TCC GCG TTA CAT AAC TTA CGG TAA ATG GCC CGC CTG GCT GAC CGC
TCA AGG CGC AAT GTA TTG AAT GCC ATT TAC CGG GCG GAC CGA CTG GCG

        5580          5590          5600          5610          5620
          •            •            •            •            •
CCA ACG ACC CCC GCC CAT TGA CGT CAA TAA TGA CGT ATG TTC CCA TAG
GGT TGC TGG GGG CGG GTA ACT GCA GTT ATT ACT GCA TAC AAG GGT ATC
```

FIG. 4 L

```
            5630           5640           5650           5660           5670
             •              •              •              •              •
    TAA CGC CAA TAG GGA CTT TCC ATT GAC GTC AAT GGG TGG AGT ATT TAC
    ATT GCG GTT ATC CCT GAA AGG TAA CTG CAG TTA CCC ACC TCA TAA ATG


                5680           5690           5700           5710
                 •              •              •              •
    GGT AAA CTG CCC ACT TGG CAG TAC ATC AAG TGT ATC ATA TGC CAA GTA
    CCA TTT GAC GGG TGA ACC GTC ATG TAG TTC ACA TAG TAT ACG GTT CAT


    5720           5730           5740           5750           5760
     •              •              •              •              •
    CGC CCC CTA TTG ACG TCA ATG ACG GTA AAT GGC CCG CCT GGC ATT ATG
    GCG GGG GAT AAC TGC AGT TAC TGC CAT TTA CCG GGC GGA CCG TAA TAC


        5770           5780           5790           5800           5810
         •              •              •              •              •
    CCC AGT ACA TGA CCT TAT GGG ACT TTC CTA CTT GGC AGT ACA TCT ACG
    GGG TCA TGT ACT GGA ATA CCC TGA AAG GAT GAA CCG TCA TGT AGA TGC


            5820           5830           5840           5850           5860
             •              •              •              •              •
    TAT TAG TCA TCG CTA TTA CCA TGG TGA TGC GGT TTT GGC AGT ACA TCA
    ATA ATC AGT AGC GAT AAT GGT ACC ACT ACG CCA AAA CCG TCA TGT AGT


            5870           5880           5890           5900           5910
             •              •              •              •              •
    ATG GGC GTG GAT AGC GGT TTG ACT CAC GGG GAT TTC CAA GTC TCC ACC
    TAC CCG CAC CTA TCG CCA AAC TGA GTG CCC CTA AAG GTT CAG AGG TGG


                5920           5930           5940           5950
                 •              •              •              •
    CCA TTG ACG TCA ATG GGA GTT TGT TTT GGC ACC AAA ATC AAC GGG ACT
    GGT AAC TGC AGT TAC CCT CAA ACA AAA CCG TGG TTT TAG TTG CCC TGA


    5960           5970           5980           5990           6000
     •              •              •              •              •
    TTC CAA AAT GTC GTA ACA ACT CCG CCC CAT TGA CGC AAA TGG GCG GTA
    AAG GTT TTA CAG CAT TGT TGA GGC GGG GTA ACT GCG TTT ACC CGC CAT


        6010           6020           6030           6040           6050
         •              •              •              •              •
    GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT TAG TGA ACC
    CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA ATC ACT TGG


        6060           6070           6080           6090           6100
         •              •              •              •              •
    GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA
    CAG TCT AGC GGA CCT CTG CGG TAG GTG CGA CAA AAC TGG AGG TAT CTT


        6110           6120           6130           6140           6150
         •              •              •              •              •
    GAC ACC GGG ACC GAT CCA GCC TCC GCG GCC GGG AAC GGT GCA TTG GAA
    CTG TGG CCC TGG CTA GGT CGG AGG CGC CGG CCC TTG CCA CGT AAC CTT
```

FIG. 4 M

```
           6160          6170          6180          6190
            •             •             •             •
CGC GGA TTC CCC GTG CCA AGA GTG ACG TAA GTA CCG CCT ATA GAG TCT
GCG CCT AAG GGG CAC GGT TCT CAC TGC ATT CAT GGC GGA TAT CTC AGA

     6200          6210          6220          6230          6240
      •             •             •             •             •
ATA GGC CCA CCC CCT TGG CTT CTT ATG CAT GCT ATA CTG TTT TTG GCT
TAT CCG GGT GGG GGA ACC GAA GAA TAC GTA CGA TAT GAC AAA AAC CGA

        6250          6260          6270          6280          6290
         •             •             •             •             •
TGG GGT CTA TAC ACC CCC GCT TCC TCA TGT TAT AGG TGA TGG TAT AGC
ACC CCA GAT ATG TGG GGG CGA AGG AGT ACA ATA TCC ACT ACC ATA TCG

        6300          6310          6320          6330          6340
         •             •             •             •             •
TTA GCC TAT AGG TGT GGG TTA TTG ACC ATT ATT GAC CAC TCC CCT ATT
AAT CGG ATA TCC ACA CCC AAT AAC TGG TAA TAA CTG GTG AGG GGA TAA

           6350          6360          6370          6380          6390
            •             •             •             •             •
GGT GAC GAT ACT TTC CAT TAC TAA TCC ATA ACA TGG CTC TTT GCC ACA
CCA CTG CTA TGA AAG GTA ATG ATT AGG TAT TGT ACC GAG AAA CGG TGT

              6400          6410          6420          6430
               •             •             •             •
ACT CTC TTT ATT GGC TAT ATG CCA ATA CAC TGT CCT TCA GAG ACT GAC
TGA GAG AAA TAA CCG ATA TAC GGT TAT GTG ACA GGA AGT CTC TGA CTG

     6440          6450          6460          6470          6480
      •             •             •             •             •
ACG GAC TCT GTA TTT TTA CAG GAT GGG GTC TCA TTT ATT ATT TAC AAA
TGC CTG AGA CAT AAA AAT GTC CTA CCC CAG AGT AAA TAA TAA ATG TTT

        6490          6500          6510          6520          6530
         •             •             •             •             •
TTC ACA TAT ACA ACA CCA CCG TCC CCA GTG CCC GCA GTT TTT ATT AAA
AAG TGT ATA TGT TGT GGT GGC AGG GGT CAC GGG CGT CAA AAA TAA TTT

        6540          6550          6560          6570          6580
         •             •             •             •             •
CAT AAC GTG GGA TCT CCA CGC GAA TCT CGG GTA CGT GTT CCG GAC ATG
GTA TTG CAC CCT AGA GGT GCG CTT AGA GCC CAT GCA CAA GGC CTG TAC

        6590          6600          6610          6620          6630
         •             •             •             •             •
GGC TCT TCT CCG GTA GCG GCG GAG CTT CTA CAT CCG AGC CCT GCT CCC
CCG AGA AGA GGC CAT CGC CGC CTC GAA GAT GTA GGC TCG GGA CGA GGG

        6640          6650          6660          6670
         •             •             •             •
ATG CCT CCA GCG ACT CAT GGT CGC TCG GCA GCT CCT TGC TCC TAA CAG
TAC GGA GGT CGC TGA GTA CCA GCG AGC CGT CGA GGA ACG AGG ATT GTC
```

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FIG. 4 N

```
6680          6690          6700          6710          6720
  •             •             •             •             •
TGG AGG CCA GAC TTA GGC ACA GCA CGA TGC CCA CCA CCA CCA GTG TGC
ACC TCC GGT CTG AAT CCG TGT CGT GCT ACG GGT GGT GGT GGT CAC ACG

      6730          6740          6750          6760          6770
        •             •             •             •             •
CGC ACA AGG CCG TGG CGG TAG GGT ATG TGT CTG AAA ATG AGC TCG GGG
GCG TGT TCC GGC ACC GCC ATC CCA TAC ACA GAC TTT TAC TCG AGC CCC

        6780          6790          6800          6810          6820
          •             •             •·            •             •
AGC GGG CTT GCA CCG CTG ACG CAT TTG GAA GAC TTA AGG CAG CGG CAG
TCG CCC GAA CGT GGC GAC TGC GTA AAC CTT CTG AAT TCC GTC GCC GTC

        6830          6840          6850          6860          6870
          •             •             •             •             •
AAG AAG ATG CAG GCA GCT GAG TTG TTG TGT TCT GAT AAG AGT CAG AGG
TTC TTC TAC GTC CGT CGA CTC AAC AAC ACA AGA CTA TTC TCA GTC TCC

          6880          6890          6900          6910
            •             •             •             •
TAA CTC CCG TTG CGG TGC TGT TAA CGG TGG AGG GCA GTG TAG TCT GAG
ATT GAG GGC AAC GCC ACG ACA ATT GCC ACC TCC CGT CAC ATC AGA CTC

6920          6930          6940          6950          6960
  •             •             •             •             •
CAG TAC TCG TTG CTG CCG CGC GCG CCA CCA GAC ATA ATA GCT GAC AGA
GTC ATG AGC AAC GAC GGC GCG CGC GGT GGT CTG TAT TAT CGA CTG TCT

    6970          6980          6990          7000          7010
      •             •             •             •             •
CTA ACA GAC TGT TCC TTT CCA TGG GTC TTT TCT GCA GTC ACC GTC CTT
GAT TGT CTG ACA AGG AAA GGT ACC CAG AAA AGA CGT CAG TGG CAG GAA

    7020          7030          7040          7050          7060
      •             •             •             •             •
GAC ACG AAG CTT GGG CTG CAG GTC GAT CGA CTC TAG AGG ATC GAT CCC
CTG TGC TTC GAA CCC GAC GTC CAG CTA GCT GAG ATC TCC TAG CTA GGG

      7070
        •
CGG GCG AGC TC
GCC CGC TCG AG
```

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FIG. 5 A

The pEe12TF8LCDR3 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted LC gene, TF8LCDR3, are translated.

Sequence Range: 1 to 7864

```
            10           20           30           40           50
             •            •            •            •            •
AAT TCA CC ATG GGT GTG CCA ACT CAG GTA TTA GGA TTA CTG CTG CTG TGG
TTA AGT GG TAC CCA CAC GGT TGA GTC CAT AAT CCT AAT GAC GAC GAC ACC
          Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp>

            60           70           80           90
             •            •            •            •
CTT ACA GAT GCA AGA TGT GAT ATC CAA ATG ACA CAA TCT CCT TCT TCT
GAA TGT CTA CGT TCT ACA CTA TAG GTT TAC TGT GTT AGA GGA AGA AGA
Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser>

  100         110          120          130          140
   •           •            •            •            •
CTA AGT GCT TCT GTC GGA GAT AGA GTA ACA ATT ACA TGT AAG GCG AGT
GAT TCA CGA AGA CAG CCT CTA TCT CAT TGT TAA TGT ACA TTC CGC TCA
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser>

     150          160          170          180          190
      •            •            •            •            •
CAG GAC ATT AGA AAG TAT TTA AAC TGG TAT CAG CAA AAA CCT GGG AAG
GTC CTG TAA TCT TTC ATA AAT TTG ACC ATA GTC GTT TTT GGA CCC TTC
Gln Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys>

     200          210          220          230          240
      •            •            •            •            •
GCT CCT AAG CTA CTG ATT TAT TAT GCA ACA AGT TTC GCA GAT GGA GTA
CGA GGA TTC GAT GAC TAA ATA ATA CGT TGT TCA AAC CGT CTA CCT CAT
Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val>

     250          260          270          280          290
      •            •            •            •            •
CCT TCT AGA TTT TCT GGT TCT GGC TCT GGA ACA GAC TAC ACA TTC ACA
GGA AGA TCT AAA AGA CCA AGA CCG AGA CCT TGT CTG ATG TGT AAG TGT
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr>

     300          310          320          330
      •            •            •            •
ATT TCT TCT CTC CAA CCT GAG GAC ATT GCT ACA TAC TAC TGC CTA CAA
TAA AGA AGA GAG GTT GGA CTC CTG TAA CGA TGT ATG ATG ACG GAT GTT
Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln>

  340         350          360          370          380
   •           •            •            •            •
CAT GGT GAG AGT CCG TAT ACA TTT GGA CAA GGA ACA AAA CTA GAG ATC
GTA CCA CTC TCA GGC ATA TGT AAA CCT GTT CCT TGT TTT GAT CTC TAG
His Gly Glu Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile>

  390         400          410          420          430
   •           •            •            •            •
ACA AGA ACT GTT GCG GCG CCG TCT GTC TTC ATC TTC CCG CCA TCT GAT
TGT TCT TGA CAA CGC CGC GGC AGA CAG AAG TAG AAG GGC GGT AGA CTA
Thr Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp>
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RECTIFIED SHEET (RULE 91)

ISA/EP

FIG. 5 B

```
        440           450           460           470           480
         •             •             •             •             •
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC
CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn>

        490           500           510           520           530
         •             •             •             •             •
TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC
AAG ATA GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu>

        540           550           560           570
         •             •             •             •
CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC
GTT AGC CCA TTG AGG GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp>

580           590           600           610           620
 •             •             •             •             •
AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC
TCG TGG ATG TCG GAG TCG TCG TGG GAC TGC GAC TCG TTT CGT CTG ATG
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr>

        630           640           650           660           670
         •             •             •             •             •
GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG GTA GTC CCG GAC TCG
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser>

        680           690           700           710           720
         •             •             •             •             •
TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT T AGA GGG AGA AGT
AGC GGG CAG TGT TTC TCG AAG TTG TCC CCT CTC ACA A TCT CCC TCT TCA
Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys>

        730           740           750           760           770
         •             •             •             •             •
GCC CCC ACC TGC TCC TCA GTT CCA GCC TGG GGA TCA TAA TCA GCC ATA
CGG GGG TGG ACG AGG AGT CAA GGT CGG ACC CCT AGT ATT AGT CGG TAT

        780           790           800           810
         •             •             •             •
CCA CAT TTG TAG AGG TTT TAC TTG CTT TAA AAA ACC TCC CAC ACC TCC
GGT GTA AAC ATC TCC AAA ATG AAC GAA ATT TTT TGG AGG GTG TGG AGG

820           830           840           850           860
 •             •             •             •             •
CCC TGA ACC TGA AAC ATA AAA TGA ATG CAA TTG TTG TTG TTA ACT TGT
GGG ACT TGG ACT TTG TAT TTT ACT TAC GTT AAC AAC AAC AAT TGA ACA

870           880           890           900           910
 •             •             •             •             •
TTA TTG CAG CTT ATA ATG GTT ACA AAT AAA GCA ATA GCA TCA CAA ATT
AAT AAC GTC GAA TAT TAC CAA TGT TTA TTT CGT TAT CGT AGT GTT TAA
```

FIG. 5 C

```
       920             930             940             950             960
        •               •               •               •               •
TCA CAA ATA AAG CAT TTT TTT CAC TGC ATT CTA GTT GTG GTT TGT CCA
AGT GTT TAT TTC GTA AAA AAA GTG ACG TAA GAT CAA CAC CAA ACA GGT


              970             980             990            1000            1010
               •               •               •               •               •
AAC TCA TCA ATG TAT CTT ATC ATG TCT GGA TCC TCT ACG CCG GAC GCA
TTG AGT AGT TAC ATA GAA TAG TAC AGA CCT AGG AGA TGC GGC CTG CGT


         1020            1030            1040            1050
          •               •               •               •
TCG TGG CCG GCA TCA CCG GCG CCA CAG GTG CGG TTG CTG GCG CCT ATA
AGC ACC GGC CGT AGT GGC CGC GGT GTC CAC GCC AAC GAC CGC GGA TAT


  1060           1070            1080            1090            1100
   •               •               •               •               •
TCG CCG ACA TCA CCG ATG GGG AAG ATC GGG CTC GCC ACT TCG GGC TCA
AGC GGC TGT AGT GGC TAC CCC TTC TAG CCC GAG CGG TGA AGC CCG AGT


  1110           1120            1130            1140            1150
   •               •               •               •               •
TGA GCG CTT GTT TCG GCG TGG GTA TGG TGG CAG GCC CGT GGC CGG GGG
ACT CGC GAA CAA AGC CGC ACC CAT ACC ACC GTC CGG GCA CCG GCC CCC


         1160            1170            1180            1190            1200
          •               •               •               •               •
ACT GTT GGG CGC CAT CTC CTT GCA TGC ACC ATT CCT TGC GGC GGC GGT
TGA CAA CCC GCG GTA GAG GAA CGT ACG TGG TAA GGA ACG CCG CCG CCA


         1210            1220            1230            1240            1250
          •               •               •               •               •
GCT CAA CGG CCT CAA CCT ACT ACT GGG CTG CTT CCT AAT GCA GGA GTC
CGA GTT GCC GGA GTT GGA TGA TGA CCC GAC GAA GGA TTA CGT CCT CAG


         1260            1270            1280            1290
          •               •               •               •
GCA TAA GGG AGA GCG TCG ACC TCG GGC CGC GTT GCT GGC GTT TTT CCA
CGT ATT CCC TCT CGC AGC TGG AGC CCG GCG CAA CGA CCG CAA AAA GGT


  1300           1310            1320            1330            1340
   •               •               •               •               •
TAG GCT CCG CCC CCC TGA CGA GCA TCA CAA AAA TCG ACG CTC AAG TCA
ATC CGA GGC GGG GGG ACT GCT CGT AGT GTT TTT AGC TGC GAG TTC AGT


  1350           1360            1370            1380            1390
   •               •               •               •               •
GAG GTG GCG AAA CCC GAC AGG ACT ATA AAG ATA CCA GGC GTT TCC CCC
CTC CAC CGC TTT GGG CTG TCC TGA TAT TTC TAT GGT CCG CAA AGG GGG


         1400            1410            1420            1430            1440
          •               •               •               •               •
TGG AAG CTC CCT CGT GCG CTC TCC TGT TCC GAC CCT GCC GCT TAC CGG
ACC TTC GAG GGA GCA CGC GAG AGG ACA AGG CTG GGA CGG CGA ATG GCC


         1450            1460            1470            1480            1490
          •               •               •               •               •
ATA CCT GTC CGC CTT TCT CCC TTC GGG AAG CGT GGC GCT TTC TCA ATG
TAT GGA CAG GCG GAA AGA GGG AAG CCC TTC GCA CCG CGA AAG AGT TAC
```

FIG. 5 D

```
         1500         1510         1520         1530
          •            •            •            •
  CTC ACG CTG TAG GTA TCT CAG TTC GGT GTA GGT CGT TCG CTC CAA GCT
  GAG TGC GAC ATC CAT AGA GTC AAG CCA CAT CCA GCA AGC GAG GTT CGA

1540       1550         1560         1570       1580
  •          •            •            •          •
  GGG CTG TGT GCA CGA ACC CCC CGT TCA GCC CGA CCG CTG CGC CTT ATC
  CCC GAC ACA CGT GCT TGG GGG GCA AGT CGG GCT GGC GAC GCG GAA TAG

    1590         1600         1610         1620         1630
     •            •            •            •            •
  CGG TAA CTA TCG TCT TGA GTC CAA CCC GGT AAG ACA CGA CTT ATC GCC
  GCC ATT GAT AGC AGA ACT CAG GTT GGG CCA TTC TGT GCT GAA TAG CGG

     1640         1650         1660         1670         1680
      •            •            •            •            •
  ACT GGC AGC AGC CAC TGG TAA CAG GAT TAG CAG AGC GAG GTA TGT AGG
  TGA CCG TCG TCG GTG ACC ATT GTC CTA ATC GTC TCG CTC CAT ACA TCC

       1690         1700         1710         1720         1730
        •            •            •            •            •
  CGG TGC TAC AGA GTT CTT GAA GTG GTG GCC TAA CTA CGG CTA CAC TAG
  GCC ACG ATG TCT CAA GAA CTT CAC CAC CGG ATT GAT GCC GAT GTG ATC

         1740         1750         1760         1770
          •            •            •            •
  AAG GAC AGT ATT TGG TAT CTG CGC TCT GCT GAA GCC AGT TAC CTT CGG
  TTC CTG TCA TAA ACC ATA GAC GCG AGA CGA CTT CGG TCA ATG GAA GCC

1780       1790         1800         1810         1820
  •          •            •            •            •
  AAA AAG AGT TGG TAG CTC TTG ATC CGG CAA ACA AAC CAC CGC TGG TAG
  TTT TTC TCA ACC ATC GAG AAC TAG GCC GTT TGT TTG GTG GCG ACC ATC

1830         1840         1850         1860       1870
  •            •            •            •          •
  CGG TGG TTT TTT TGT TTG CAA GCA GCA GAT TAC GCG CAG AAA AAA AGG
  GCC ACC AAA AAA ACA AAC GTT CGT CGT CTA ATG CGC GTC TTT TTT TCC

    1880         1890         1900         1910         1920
     •            •            •            •            •
  ATC TCA AGA AGA TCC TTT GAT CTT TTC TAC GGG GTC TGA CGC TCA GTG
  TAG AGT TCT TCT AGG AAA CTA GAA AAG ATG CCC AGT ACT GCG AGT CAC

      1930         1940         1950         1960         1970
       •            •            •            •            •
  GAA CGA AAA CTC ACG TTA AGG GAT TTT GGT CAT GAG ATT ATC AAA AAG
  CTT GCT TTT GAG TGC AAT TCC CTA AAA CCA GTA CTC TAA TAG TTT TTC

       1980         1990         2000         2010
        •            •            •            •
  GAT CTT CAC CTA GAT CCT TTT AAA TTA AAA ATG AAG TTT TAA ATC AAT
  CTA GAA GTG GAT CTA GGA AAA TTT AAT TTT TAC TTC AAA ATT TAG TTA

2020         2030         2040         2050         2060
  •            •            •            •            •
  CTA AAG TAT ATA TGA GTA AAC TTG GTC TGA CAG TTA CCA ATG CTT AAT
  GAT TTC ATA TAT ACT CAT TTG AAC CAG ACT GTC AAT GGT TAC GAA TTA
```

FIG. 5 E

```
      2070          2080          2090          2100          2110
       •             •             •             •             •
  CAG TGA GGC ACC TAT CTC AGC GAT CTG TCT ATT TCG TTC ATC CAT AGT
  GTC ACT CCG TGG ATA GAG TCG CTA GAC AGA TAA AGC AAG TAG GTA TCA

        2120          2130          2140          2150          2160
         •             •             •             •             •
  TGC CTG ACT CCC CGT CGT GTA GAT AAC TAC GAT ACG GGA GGG CTT ACC
  ACG GAC TGA GGG GCA GCA CAT CTA TTG ATG CTA TGC CCT CCC GAA TGG

         2170          2180          2190          2200          2210
          •             •             •             •             •
  ATC TGG CCC CAG TGC TGC AAT GAT ACC GCG AGA CCC ACG CTC ACC GGC
  TAG ACC GGG GTC ACG ACG TTA CTA TGG CGC TCT GGG TGC GAG TGG CCG

          2220          2230          2240          2250
           •             •             •             •
  TCC AGA TTT ATC AGC AAT AAA CCA GCC AGC CGG AAG GGC CGA GCG CAG
  AGG TCT AAA TAG TCG TTA TTT GGT CGG TCG GCC TTC CCG GCT CGC GTC

  2260          2270          2280          2290          2300
   •             •             •             •             •
  AAG TGG TCC TGC AAC TTT ATC CGC CTC CAT CCA GTC TAT TAA TTG TTG
  TTC ACC AGG ACG TTG AAA TAG GCG GAG GTA GGT CAG ATA ATT AAC AAC

  2310          2320          2330          2340          2350
   •             •             •             •             •
  CCG GGA AGC TAG AGT AAG TAG TTC GCC AGT TAA TAG TTT GCG CAA CGT
  GGC CCT TCG ATC TCA TTC ATC AAG CGG TCA ATT ATC AAA CGC GTT GCA

    2360          2370          2380          2390          2400
     •             •             •             •             •
  TGT TGC CAT TGC TAC AGG CAT CGT GGT GTC ACG CTC GTC GTT TGG TAT
  ACA ACG GTA ACG ATG TCC GTA GCA CCA CAG TGC GAG CAG CAA ACC ATA

         2410          2420          2430          2440          2450
          •             •             •             •             •
  GGC TTC ATT CAG CTC CGG TTC CCA ACG ATC AAG GCG AGT TAC ATG ATC
  CCG AAG TAA GTC GAG GCC AAG GGT TGC TAG TTC CGC TCA ATG TAC TAG

          2460          2470          2480          2490
           •             •             •             •
  CCC CAT GTT GTG CAA AAA AGC GGT TAG CTC CTT CGG TCC TCC GAT CGT
  GGG GTA CAA CAC GTT TTT TCG CCA ATC GAG GAA GCC AGG AGG CTA GCA

  2500          2510          2520          2530          2540
   •             •             •             •             •
  TGT CAG AAG TAA GTT GGC CGC AGT GTT ATC ACT CAT GGT TAT GGC AGC
  ACA GTC TTC ATT CAA CCG GCG TCA CAA TAG TGA GTA CCA ATA CCG TCG

  2550          2560          2570          2580          2590
   •             •             •             •             •
  ACT GCA TAA TTC TCT TAC TGT CAT GCC ATC CGT AAG ATG CTT TTC TGT
  TGA CGT ATT AAG AGA ATG ACA GTA CGG TAG GCA TTC TAC GAA AAG ACA

  2600          2610          2620          2630          2640
   •             •             •             •             •
  GAC TGG TGA GTA CTC AAC CAA GTC ATT CTG AGA ATA GTG TAT GCG GCG
  CTG ACC ACT CAT GAG TTG GTT CAG TAA GAC TCT TAT CAC ATA CGC CGC
```

FIG. 5 F

```
          2650              2660              2670              2680              2690
           •                 •                 •                 •                 •
ACC GAG TTG CTC TTG CCC GGC GTC AAC ACG GGA TAA TAC CGC GCC ACA
TGG CTC AAC GAG AAC GGG CCG CAG TTG TGC CCT ATT ATG GCG CGG TGT

          2700              2710              2720              2730
           •                 •                 •                 •
TAG CAG AAC TTT AAA AGT GCT CAT CAT TGG AAA ACG TTC TTC GGG GCG
ATC GTC TTG AAA TTT TCA CGA GTA GTA ACC TTT TGC AAG AAG CCC CGC

  2740            2750              2760              2770              2780
   •               •                 •                 •                 •
AAA ACT CTC AAG GAT CTT ACC GCT GTT GAG ATC CAG TTC GAT GTA ACC
TTT TGA GAG TTC CTA GAA TGG CGA CAA CTC TAG GTC AAG CTA CAT TGG

  2790            2800              2810              2820              2830
   •               •                 •                 •                 •
CAC TCG TGC ACC CAA CTG ATC TTC AGC ATC TTT. TAC TTT CAC CAG CGT
GTG AGC ACG TGG GTT GAC TAG AAG TCG TAG AAA ATG AAA GTG GTC GCA

          2840              2850              2860              2870              2880
           •                 •                 •                 •                 •
TTC TGG GTG AGC AAA AAC AGG AAG GCA AAA TGC CGC AAA AAA GGG AAT
AAG ACC CAC TCG TTT TTG TCC TTC CGT TTT ACG GCG TTT TTT CCC TTA

               2890            .2900              2910              2920              2930
                •               •                 •                 •                 •
AAG GGC GAC ACG GAA ATG TTG AAT ACT CAT ACT CTT CCT TTT TCA ATA
TTC CCG CTG TGC CTT TAC AAC TTA TGA GTA TGA GAA GGA AAA AGT TAT

               2940              2950              2960              2970
                •                 •                 •                 •
TTA TTG AAG CAT TTA TCA GGG TTA TTG TCT CAT GAG CGG ATA CAT ATT
AAT AAC TTC GTA AAT AGT CCC AAT AAC AGA GTA CTC GCC TAT GTA TAA

  2980            2990              3000              3010              3020
   •               •                 •                 •                 •
TGA ATC TAT TTA GAA AAA TAA ACA AAT AGG GGT TCC GCG CAC ATT TCC
ACT TAC ATA AAT CTT TTT ATT TGT TTA TCC CCA AGG CGC GTG TAA AGG

  3030              3040              3050              3060              3070
   •                 •                 •                 •                 •
CCG AAA AGT GCC ACC TGA CGT CTA AGA AAC CAT TAT TAT CAT GAC ATT
GGC TTT TCA CGG TGG ACT GCA GAT TCT TTG GTA ATA ATA GTA CTG TAA

     3080            3090              3100              3110              3120
      •               •                 •                 •                 •
AAC CTA TAA AAA TAG GCG TAT CAC GAG GCC CTG ATG GCT CTT TGC GGC
TTG GAT ATT TTT ATC CGC ATA GTG CTC CGG GAC TAC CGA GAA ACG CCG

          3130              3140              3150              3160              3170
           •                 •                 •                 •                 •
ACC CAT CGT TCG TAA TGT TCC GTG GCA CCG AGG ACA ACC CTC AAG AGA
TGG GTA GCA AGC ATT ACA AGG CAC CGT GGC TCC TGT TGG GAG TTC TCT

          3180              3190              3200              3210
           •                 •                 •                 •
AAA TGT AAT CAC ACT GGC TCA CCT TCG GGT GGG CCT TTC TGC GTT TAT
TTT ACA TTA GTG TGA CCG AGT GGA AGC CCA CCC GGA AAG ACG CAA ATA
```

FIG. 5 G

```
    3220         3230         3240         3250         3260
     •            •            •            •            •
  AAG GAG ACA CTT TAT GTT TAA GAA GGT TGG TAA ATT CCT TGC GGC TTT
  TTC CTC TGT GAA ATA CAA ATT CTT CCA ACC ATT TAA GGA ACG CCG AAA

    3270         3280         3290         3300         3310
     •            •            •            •            •
  GGC AGC CAA GCT AGA GAT CCG GCT GTG GAA TGT GTG TCA GTT AGG GTG
  CCG TCG GTT CGA TCT CTA GGC CGA CAC CTT ACA CAC AGT CAA TCC CAC

       3320         3330         3340         3350         3360
        •            •            •            •            •
     TGG AAA GTC CCC AGG CTC CCC AGC AGG CAG AAG TAT GCA AAG CAT GCA
     ACC TTT CAG GGG TCC GAG GGG TCG TCC GTC TTC ATA CGT TTC GTA CGT

        3370         3380         3390         3400         3410
         •            •            •            •            •
      TCT CAA TTA GTC AGC AAC CAG GCT CCC CAG CAG GCA GAA GTA TGC AAA
      AGA GTT AAT CAG TCG TTG GTC CGA GGG GTC GTC CGT CTT CAT ACG TTT

          3420         3430         3440         3450
           •            •            •            •
       GCA TGC ATC TCA ATT AGT CAG CAA CCA TAG TCC CGC CCC TAA CTC CGC
       CGT ACG TAG AGT TAA TCA GTC GTT GGT ATC AGG GCG GGG ATT GAG GCG

    3460         3470         3480         3490         3500
     •            •            •            •            •
  CCA TCC CGC CCC TAA CTC CGC CCA GTT CCG CCC ATT CTC CGC CCC ATG
  GGT AGG GCG GGG ATT GAG GCG GGT CAA GGC GGG TAA GAG GCG GGG TAC

    3510         3520         3530         3540         3550
     •            •            •            •            •
  GCT GAC TAA TTT TTT TTA TTT ATG CAG AGG CCG AGG CCG CCT CGG CCT
  CGA CTG ATT AAA AAA AAT AAA TAC GTC TCC GGC TCC GGC GGA GCC GGA

       3560         3570         3580         3590         3600
        •            •            •            •            •
     CTG AGC TAT TCC AGA AGT AGT GAG GAG GCT TTT TTG GAG GCC TAG GCT
     GAC TCG ATA AGG TCT TCA TCA CTC CTC CGA AAA AAC CTC CGG ATC CGA

          3610         3620         3630         3640         3650
           •            •            •            •            •
       TTT GCA AAA AGC TAG CTT GGG GCC ACC GCT CAG AGC ACC TTC CAC CAT
       AAA CGT TTT TCG ATC GAA CCC CGG TGG CGA GTC TCG TGG AAG GTG GTA

          3660         3670         3680         3690
           •            •            •            •
       GGC CAC CTC AGC AAG TTC CCA CTT GAA CAA AAA CAT CAA GCA AAT GTA
       CCG GTG GAG TCG TTC AAG GGT GAA CTT GTT TTT GTA GTT CGT TTA CAT

    3700         3710         3720         3730         3740
     •            •            •            •            •
  CTT GTG CCT GCC CCA GGG TGA GAA AGT CCA AGC CAT GTA TAT CTG GGT
  GAA CAC GGA CGG GGT CCC ACT CTT TCA GGT TCG GTA CAT ATA GAC CCA

    3750         3760         3770         3780         3790
     •            •            •            •            •
  TGA TGG TAC TGG AGA AGG ACT GCG CTG CAA AAC CCG CAC CCT GGA CTG
  ACT ACC ATG ACC TCT TCC TGA CGC GAC GTT TTG GGC GTG GGA CCT GAC
```

FIG. 5 H

```
     3800           3810          3820          3830          3840
      •              •             •             •             •
TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC
ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG


        3850          3860          3870          3880          3890
         •             •             •             •             •
TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC
ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG


          3900          3910          3920          3930
           •             •             •             •
TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT
ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA


3940          3950          3960          3970          3980
  •             •             •             •             •
GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT
CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CGG ACG TCT CTG GTT AAA


3990          4000          4010          4020          4030
  •             •             •             •             •
AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC
TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG


          4040          4050          4060          4070          4080
           •             •             •             •             •
CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA
GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT


          4090          4100          4110          4120          4130
           •             •             •             •             •
CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA
GGG AAA ACC AAC CGG AAG GTT ACC GAA AGG ACC CGG GGT TCC AGG CAT


            4140          4150          4160          4170
             •             •             •             •
TTA CTG TGG TGT GGG CGC AGA CAA AGC CTA TCG CAG GGA TAT CGT GGA
AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT


4180          4190          4200          4210          4220
  •             •             •             •             •
GGC TCA CTA CCG CGC CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC
CCG AGT GAT GGC GCG GAC GAA CAT ACG ACC CCA GTT CTA ATG TCC TTG


4230          4240          4250          4260          4270
  •             •             •             •             •
AAA TGC TGA GGT CAT GCC TGC CCA GTG GGA ACT CCA AAT AGG ACC CTG
TTT ACG ACT CCA GTA CGG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC


          4280          4290          4300          4310          4320
           •             •             •             •             •
TGA AGG AAT CCG CAT GGG AGA TCA TCT CTC GGT GGC CCG TTT CAT CTT
ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA


          4330          4340          4350          4360          4370
           •             •             •             •             •
NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA
NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT
```

FIG. 5 I

```
        4380            4390            4400          4410
         •               •               •             •
GCC CAT TCC TGG GAA CTG GAA TGG TGC AGG CTG CCA TAC CAA CTT TAG
CGG GTA AGG ACC CTT GAC CTT ACC ACG TCC GAC GGT ATG GTT GAA ATC

  4420          4430            4440          4450          4460
    •             •               •             •             •
CAC CAA GGC CAT GCG GGA GGA GAA TGG TCT GAA GCA CAT CGA GGA GGC
GTG GTT CCG GTA CGC CCT CCT CTT ACC AGA CTT CGT GTA GCT CCT CCG

    4470          4480          4490          4500          4510
      •             •             •             •             •
CAT CGA GAA ACT AAG CAA GCG GCA CCG GTA CCA CAT TCG AGC CTA CGA
GTA GCT CTT TGA TTC GTT CGC CGT GGC CAT GGT GTA AGC TCG GAT GCT

      4520          4530          4540          4550          4560
        •             •             •             •             •
TCC CAA GGG GGG CCT GGA CAA TGC CCG TGG TCT GAC TGG GTT CCA CGA
AGG GTT CCC CCC GGA CCT GTT ACG GGC ACC AGA CTG ACC CAA GGT GCT

        4570          4580          4590          4600          4610
          •             •             •             •             •
AAC GTC CAA CAT CAA CGA CTT TTC TGC TGG TGT CGC CAA TCG CAG TGC
TTG CAG GTT GTA GTT GCT GAA AAG ACG ACC ACA GCG GTT AGC GTC ACG

        4620          4630          4640          4650
          •             •             •             •
CAG CAT CCG CAT TCC CCG GAC TGT CGG CCA GGA GAA GAA AGG TTA CTT
GTC GTA GGC GTA AGG GGC CTG ACA GCC GGT CCT CTT CTT TCC AAT GAA

  4660          4670          4680          4690          4700
    •             •             •             •             •
TGA AGA CCG CGG CCC CTC TGC CAA TTG TGA CCC CTT TGC AGT GAC AGA
ACT TCT GGC GCC GGG GAG ACG GTT AAC ACT GGG GAA ACG TCA CTG TCT

    4710          4720          4730          4740          4750
      •             •             •             •             •
AGC CAT CGT CCG CAC ATC CCT TCT CAA TGA GAC TGG CCA CGA GCC CTT
TCG GTA GCA GGC GTG TAC GGA AGA GTT ACT CTG ACC GGT GCT CGG GAA

        4760          4770          4780          4790          4800
          •             •             •             •             •
CCA ATA CAA AAA CTA ATT AGA CTT TGA GTG ATC TTG AGC CTT TCC TAG
GGT TAT GTT TTT GAT TAA TCT GAA ACT CAC TAG AAC TCG GAA AGG ATC

          4810          4820          4830          4840          4850
            •             •             •             •             •
TTC ATC CCA CCC CGC CCC AGA GAG ATC TTT GTG AAG GAA CCT TAC TTC
AAG TAG GGT GGG GCG GGG TCT CTC TAG AAA CAC TTC CTT GGA ATG AAG

            4860          4870          4880          4890
              •             •             •             •
TGT GGT GTG ACA TAA TTG GAC AAA CTA CCT ACA GAG ATT TAA AGC TCT
ACA CCA CAC TGT ATT AAC CTG TTT GAT GGA TGT CTC TAA ATT TCG AGA

  4900          4910          4920          4930          4940
    •             •             •             •             •
AAG GTA AAT ATA AAA TTT TTA AGT GTA TAA TGT GTT AAA CTA CTG ATT
TTC CAT TTA TAT TTT AAA AAT TCA CAT ATT ACA CAA TTT GAT GAC TAA
```

FIG. 5 J

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      4950           4960           4970           4980           4990
       •             •              •              •              •
CTA ATT GTT TGT GTA TTT TAG ATT CCA ACC TAT GGA ACT GAT GAA TGG
GAT TAA CAA ACA CAT AAA ATC TAA GGT TGG ATA CCT TGA CTA CTT ACC

         5000           5010           5020           5030           5040
          •              •              •              •              •
GAG CAG TGG TGG AAT GCC TTT AAT GAG GAA AAC CTG TTT TGC TCA GAA
CTC GTC ACC ACC TTA CGG AAA TTA CTC CTT TTG GAC AAA ACG AGT CTT

           5050           5060           5070           5080           5090
            •              •              •              •              •
GAA ATG CCA TCT AGT GAT GAT GAG GCT ACT GCT GAC TCT CAA CAT TCT
CTT TAC GGT AGA TCA CTA CTA CTC CGA TGA CGA CTG AGA GTT GTA AGA

            5100           5110           5120           5130
             •              •              •              •
ACT CCT CCA AAA AAG AAG AGA AAG GTA GAA GAC CCC AAG GAC TTT CCT
TGA GGA GGT TTT TTC TTC TCT TTC CAT CTT CTG GGG TTC CTG AAA GGA

  5140           5150           5160           5170           5180
   •              •              •              •              •
TCA GAA TTG CTA AGT TTT TTG AGT CAT GCT GTG TTT AGT AAT AGA ACT
AGT CTT AAC GAT TCA AAA AAC TCA GTA CGA CAC AAA TCA TTA TCT TGA

 5190           5200           5210           5220           5230
  •              •              •              •              •
CTT GCT TGC TTT GCT ATT TAC ACC ACA AAG GAA AAA GCT GCA CTG CTA
GAA CGA ACG AAA CGA TAA ATG TGG TGT TTC CTT TTT CGA CGT GAC GAT

      5240           5250           5260           5270           5280
       •              •              •              •              •
TAC AAG AAA ATT ATG GAA AAA TAT TCT GTA ACC TTT ATA AGT AGG CAT
ATG TTC TTT TAA TAC CTT TTT ATA AGA CAT TGG AAA TAT TCA TCC GTA

         5290           5300           5310           5320           5330
          •              •              •              •              •
AAC AGT TAT AAT CAT AAC ATA CTG TTT TTT CTT ACT CCA CAC AGG CAT
TTG TCA ATA TTA GTA TTG TAT GAC AAA AAA GAA TGA GGT GTC TCC GTA

           5340           5350           5360           5370
            •              •              •              •
AGA GTG TCT GCT ATT AAT AAC TAT GCT CAA AAA TTG TGT ACC TTT AGC
TCT CAC AGA CGA TAA TTA TTG ATA CGA GTT TTT AAC ACA TGG AAA TCG

  5380           5390           5400           5410           5420
   •              •              •              •              •
TTT TTA ATT TGT AAA GGG GTT AAT AAG GAA TAT TTG ATG TAT AGT GCC
AAA AAT TAA ACA TTT CCC CAA TTA TTC CTT ATA AAC TAC ATA TCA CGG

  5430           5440           5450           5460           5470
   •              •              •              •              •
TTG ACT AGA GAT CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT
AAC TGA TCT CTA GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA

      5480           5490           5500           5510           5520
       •              •              •              •              •
TGC TTT AAA AAA CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT
ACG AAA TTT TTT GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA
```

FIG. 5 K

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         5530          5540          5550          5560          5570
          *             *             *             *             *
    GAA TGC AAT TGT TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA
    CTT ACG TTA ACA ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT

                5580          5590          5600          5610
                 *             *             *             *
    CAA ATA AAG CAA TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC
    GTT TAT TTC GTT ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG

     5620          5630          5640          5650          5660
      *             *             *             *             *
    ACT GCA TTC TAG TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA
    TGA CGT AAG ATC AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT

     5670          5680          5690          5700          5710
      *             *             *             *             *
    TGT CTG GAT CTC TAG CTT CGT GTC AAG GAC GGT GAC TGC AGT GAA TAA
    ACA GAC CTA GAG ATC GAA GCA CAG TTC CTG CCA CTG ACG TCA CTT ATT

           5720          5730          5740          5750          5760
            *             *             *             *             *
    TAA AAT GTG TGT TTG TCC GAA ATA CGC GTT TTG AGA TTT CTG TCG CCG
    ATT TTA CAC ACA AAC AGG CTT TAT GCG CAA AAC TCT AAA GAC AGC GGC

           5770          5780          5790          5800          5810
            *             *             *             *             *
    ACT AAA TTC ATG TCG CGC GAT AGT GGT GTT TAT CGC CGA TAG AGA TGG
    TGA TTT AAG TAC AGC GCG CTA TCA CCA CAA ATA GCG GCT ATC TCT ACC

                5820          5830          5840          5850
                 *             *             *             *
    CGA TAT TGG AAA AAT CGA TAT TTG AAA ATA TGG CAT ATT GAA AAT GTC
    GCT ATA ACC TTT TTA GCT ATA AAC TTT TAT ACC GTA TAA CTT TTA CAG

     5860          5870          5880          5890          5900
      *             *             *             *             *
    GCC GAT GTG AGT TTC TGT GTA ACT GAT ATC GCC ATT TTT CCA AAA GTG
    CGG CTA CAC TCA AAG ACA CAT TGA CTA TAG CGG TAA AAA GGT TTT CAC

     5910          5920          5930          5940          5950
      *             *             *             *             *
    ATT TTT GGG CAT ACG CGA TAT CTG GCG ATA GCG CTT ATA TCC TTT ACG
    TAA AAA CCC GTA TGC GCT ATA GAC CGC TAT CGC GAA TAT AGC AAA TGC

           5960          5970          5980          5990          6000
            *             *             *             *             *
    GGG GAT GGC GAT AGA CGA CTT TGG TGA CTT GGG CGA TTC TGT GTG TCG
    CCC CTA CCG CTA TCT GCT GAA ACC ACT GAA CCC GCT AAG ACA CAC AGC

           6010          6020          6030          6040          6050
            *             *             *             *             *
    CAA ATA TCG CAG TTT CGA TAT AGG TGA CAG ACG ATA TGA GGC TAT ATC
    GTT TAT AGC GTC AAA GCT ATA TCC ACT GTC TGC TAT ACT CCG ATA TAG

           6060          6070          6080          6090
            *             *             *             *
    GCC GAT AGA GGC GAC ATC AAG CTG GCA CAT GGC CAA TGC ATA TCG ATC
    CGG CTA TCT CCG CTG TAG TTC GAC CGT GTA CCG GTT ACG TAT AGC TAG
```

FIG. 5 L

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       6100          6110          6120          6130          6140
        •             •             •             •             •
     TAT ACA TTG AAT CAA TAT TGG CCA TTA GCC ATA TTA TTC ATT GGT TAT
     ATA TGT AAC TTA GTT ATA ACC GGT AAT CGG TAT AAT AAG TAA CCA ATA

          6150          6160          6170          6180          6190
           •             •             •             •             •
        ATA GCA TAA ATC AAT ATT GGC TAT TGG CCA TTG CAT ACG TTG TAT CCA
        TAT CGT ATT TAG TTA TAA CCG ATA ACC GGT AAC GTA TGC AAC ATA GGT

          6200          6210          6220          6230          6240
           •             •             •             •             •
        TAT CAT AAT ATG TAC ATT TAT ATT GGC TCA TGT CCA ACA TTA CCG CCA
        ATA GTA TTA TAC ATG TAA ATA TAA CCG AGT ACA GGT TGT AAT GGC GGT

             6250          6260          6270          6280          6290
              •             •             •             •             •
          TGT TGA CAT TGA TTA TTG ACT AGT TAT TAA TAG TAA TCA ATT ACG GGG
          ACA ACT GTA ACT AAT AAC TGA TCA ATA ATT ATC ATT AGT TAA TGC CCC

             6300          6310          6320          6330
              •             •             •             •
          TCA TTA GTT CAT AGC CCA TAT ATG GAG TTC CGC GTT ACA TAA CTT ACG
          AGT AAT CAA GTA TCG GGT ATA TAC CTC AAG GCG CAA TGT ATT GAA TGC

     6340          6350          6360          6370          6380
      •             •             •             •             •
     GTA AAT GGC CCG CCT GGC TCA CCG CCC AAC GAC CCC CGC CCA TTG ACG
     CAT TTA CCG GGC GGA CCG ACT GGC GGG TTG CTG GGG GCG GGT AAC TGC

     6390          6400          6410          6420          6430
      •             •             •             •             •
     TCA ATA ATG ACG TAT GTT CCC ATA GTA ACG CCA ATA GGG ACT TTC CAT
     AGT TAT TAC TGC ATA CAA GGG TAT CAT TGC GGT TAT CCC TGA AAG GTA

          6440          6450          6460          6470          6480
           •             •             •             •             •
        TGA CGT CAA TGG GTG GAG TAT TTA CGG TAA ACT GCC CAC TTG GCA GTA
        ACT GCA GTT ACC CAC CTC ATA AAT GCC ATT TGA CGG GTG AAC CGT CAT

             6490          6500          6510          6520          6530
              •             •             •             •             •
          CAT CAA GTG TAT CAT ATG CCA AGT ACG CCC CCT ATT GAC GTC AAT GAC
          GTA GTT CAC ATA GTA TAC GGT TCA TGC GGG GGA TAA CTG CAG TTA CTG

             6540          6550          6560          6570
              •             •             •             •
          GGT AAA TGG CCC GCC TGG CAT TAT GCC CAG TAC ATG ACC TTA TGG GAC
          CCA TTT ACC GGG CGG ACC GTA ATA CGG GTC ATG TAC TGG AAT ACC CTG

     6580          6590          6600          6610          6620
      •             •             •             •             •
     TTT CCT ACT TGG CAG TAC ATC TAC GTA TTA GTC ATC GCT ATT ACC ATG
     AAA GGA TGA ACC GTC ATG TAG ATG CAT AAT CAG TAG CGA TAA TGG TAC

     6630          6640          6650          6660          6670
      •             •             •             •             •
     GTG ATG CGG TTT TGG CAG TAC ATC AAT GGG CGT GGA TAG CGG TTT GAC
     CAC TAC GCC AAA ACC GTC ATG TAG TTA CCC GCA CCT ATC GCC AAA CTG
```

FIG. 5 M

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        6680            6690            6700            6710           6720
         •               •               •               •              •
     TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG
     AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC

          6730            6740            6750            6760          6770
           •               •               •               •             •
     TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC
     AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG

            6780            6790            6800            6810
             •               •               •               •
     GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CGG TGG GAG GTC TAT
     CGG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA

      6820            6830            6840            6850            6860
       •               •               •               •               •
     ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CGC CAT
     TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CGG ACC TCT GCG GTA

    6870            6880            6890            6900            6910
     •               •               •               •               •
     CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC
     GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG

          6920            6930            6940            6950            6960
           •               •               •               •               •
     CGC GGC CGG GAA CGG TGC ATT GGA ACG CGG ATT CCC CGT GCC AAG AGT
     GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CGG TTC TCA

            6970            6980            6990            7000            7010
             •               •               •               •               •
     GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT
     CTG CAT TCA TGG CGG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA

            7020            7030            7040            7050
             •               •               •               •
     TAT GCA TGC TAT ACT GTT TTT GGC TTG GGG TCT ATA CAC CCC CGC TTC
     ATA CGT ACG ATA TGA CAA AAA CCG AAC CCC AGA TAT GTG GGG GCG AAG

    7060            7070            7080            7090            7100
     •               •               •               •               •
     CTC ATC TTA TAG GTG ATC GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT
     GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA

    7110            7120            7130            7140            7150
     •               •               •               •               •
     GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA
     CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT

        7160            7170            7180            7190            7200
         •               •               •               •               •
     ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TGG CTA TAT GCC
     TAG GTA TTG TAC CGA GAA ACG GTG TTG AGA GAA ATA ACC GAT ATA CGG

        7210            7220            7230            7240            7250
         •               •               •               •               •
     AAT ACA CTG TCC TTC AGA GAC TGA CAC GGA CTC TGT ATT TTT ACA GGA
     TTA TGT GAC AGG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT
```

FIG. 5 N

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          7260            7270            7280            7290
           •               •               •               •
TGG GGT CTC ATT TAT TAT TTA CAA ATT CAC ATA TAC AAC ACC ACC GTC
ACC CCA GAG TAA ATA ATA AAT GTT TAA GTG TAT ATG TTG TGG TGG CAG

  7300            7310            7320            7330            7340
   •               •               •               •               •
CCC AGT GCC CGC AGT TTT TAT TAA ACA TAA CGT GGG ATC TCC ACG CGA
GGG TCA CGG GCG TCA AAA ATA ATT TGT ATT GCA CCC TAG AGG TGC GCT

  7350            7360            7370            7380            7390
   •               •               •               •               •
ATC TCG GGT ACG TGT TCC GGA CAT GGG CTC TTC TCC GGT AGC GGC GGA
TAG AGC CCA TGC ACA AGG CCT GTA CCC GAG AAG AGG CCA TCG CCG CCT

      7400            7410            7420            7430            7440
       •               •               •               •               •
GCT TCT ACA TCC GAG CCC TGC TCC CAT GCC TCC AGC GAC TCA TGG TCG
CGA AGA TGT AGG CTC GGG ACG AGG GTA CGG AGG TCG CTG AGT ACC AGC

      7450            7460            7470            7480            7490
       •               •               •               •               •
CTC GGC AGC TCC TTG CTC CTA ACA GTG GAG GCC AGA CTT AGG CAC AGC
GAG CCG TCG AGG AAC GAG GAT TGT CAC CTC CGG TCT GAA TCC GTG TCG

          7500            7510            7520            7530
           •               •               •               •
ACG ATG CCC ACC ACC ACC AGT GTG CCG CAC AAG GCC GTG GCG GTA GGG
TGC TAC GGG TGG TGG TGG TCA CAC GGC GTC TTC CGG CAC CGC CAT CCC

7540            7550            7560            7570            7580
 •               •               •               •               •
TAT GTG TCT GAA AAT GAG CTC CGG GAG CGG GCT TGC ACC GCT GAC GCA
ATA CAC AGA CTT TTA CTC GAG CCC CTC GCC CGA ACG TGG CGA CTG CGT

  7590            7600            7610            7620            7630
   •               •               •               •               •
TTT GGA AGA CTT AAG GCA GCG GCA GAA GAA GAT GCA CGC AGC TGA GTT
AAA CCT TCT GAA TTC CGT CGC CGT CTT CTT CTA CGT CCG TCG ACT CAA

     7640            7650            7660            7670            7680
      •               •               •               •               •
GTT GTG TTC TGA TAA GAG TCA GAG GTA ACT CCC GTT GCG GTG CTG TTA
CAA CAC AAG ACT ATT CTC AGT CTC CAT TGA GGG CAA CGC CAC GAC AAT

        7690            7700            7710            7720            7730
         •               •               •               •               •
ACG GTG GAG GGC AGT GTA GTC TGA GCA GTA CTC GTT GCT GCC GCG CGC
TGC CAC CTC CCG TCA CAT CAG ACT CGT CAT GAG CAA CGA CGG CGC GCG

          7740            7750            7760            7770
           •               •               •               •
GCC ACC AGA CAT AAT AGC TGA CAG ACT AAC AGA CTG TTC CTT TCC ATG
CGG TGG TCT GTA TTA TCG ACT GTC TGA TTG TCT GAC AAG GAA AGG TAC

7780            7790            7800            7810            7820
 •               •               •               •               •
GGT CTT TTC TGC AGT CAC CGT CCT TGA CAC GAA GCT TGG GCT GCA GGT
CCA GAA AAG ACG TCA GTG GCA GGA ACT GTG CTT CGA ACC CGA CGT CCA
```
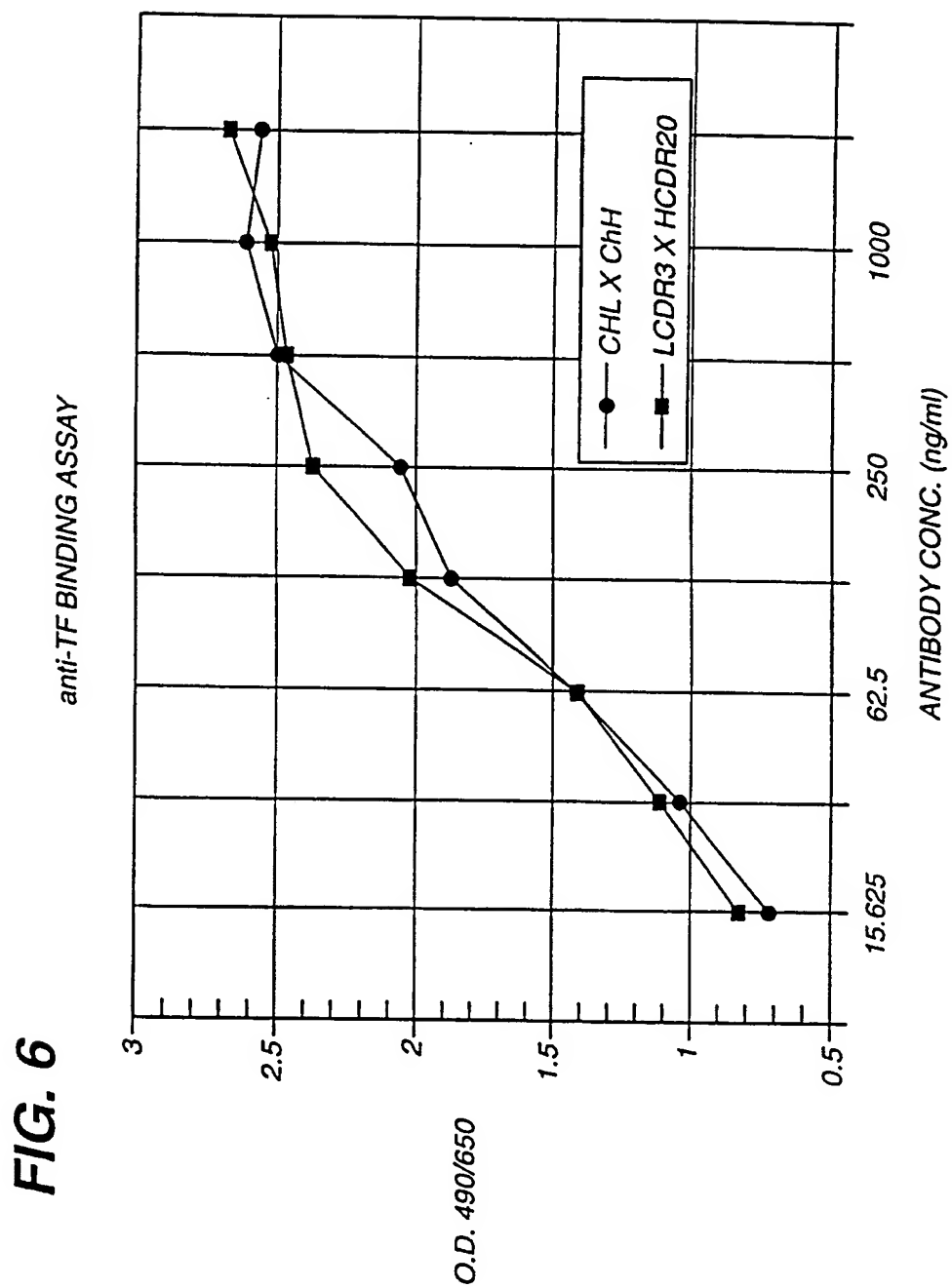
FIG. 5 O

```
        7830           7840           7850           7860
          •              •              •              •
    CGA TCG ACT CTA GAG GAT CGA TCC CCG GGC GAG CTC G
    GCT AGC TGA GAT CTC CTA GCT AGG GGC CCG CTC GAG C
```
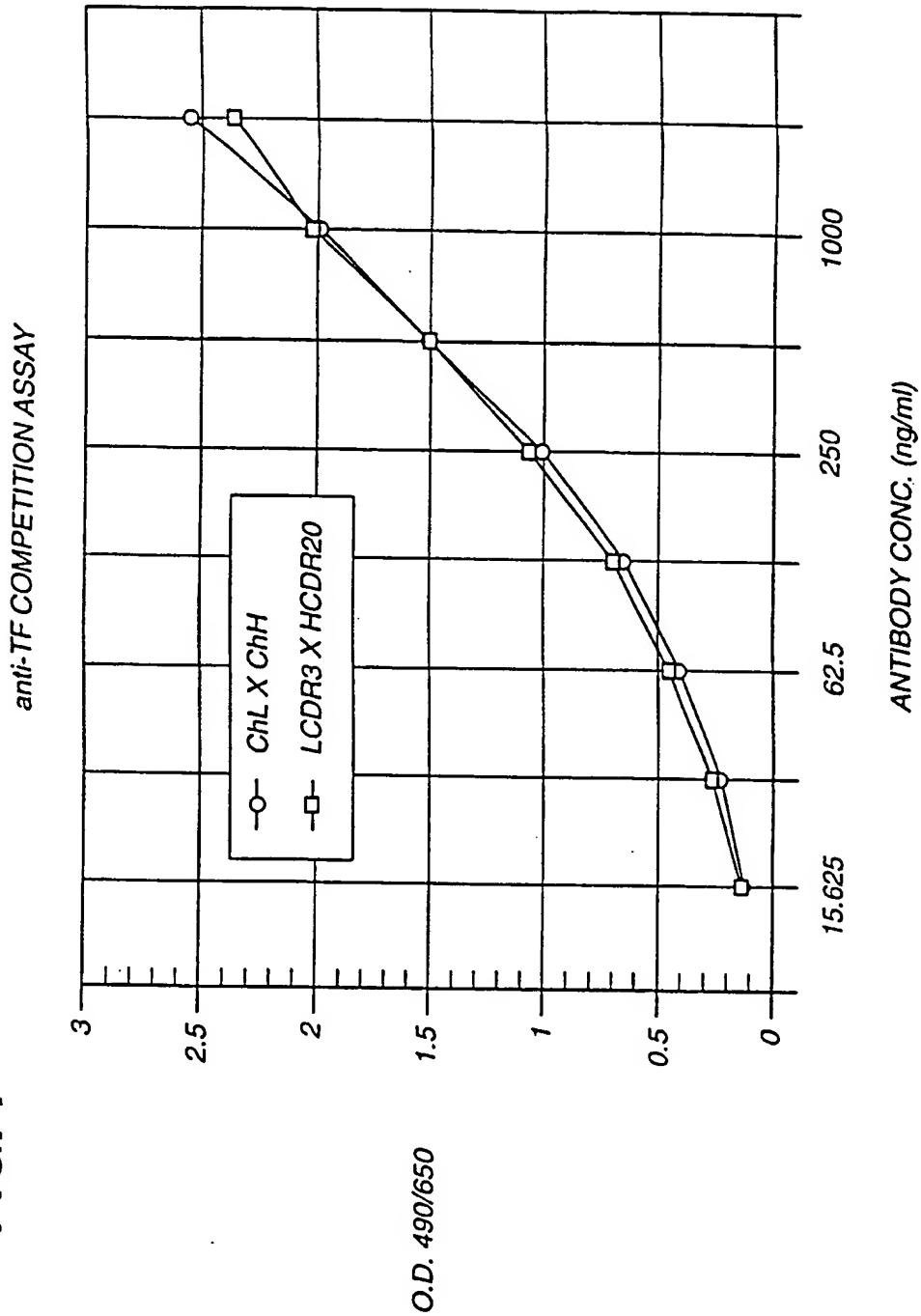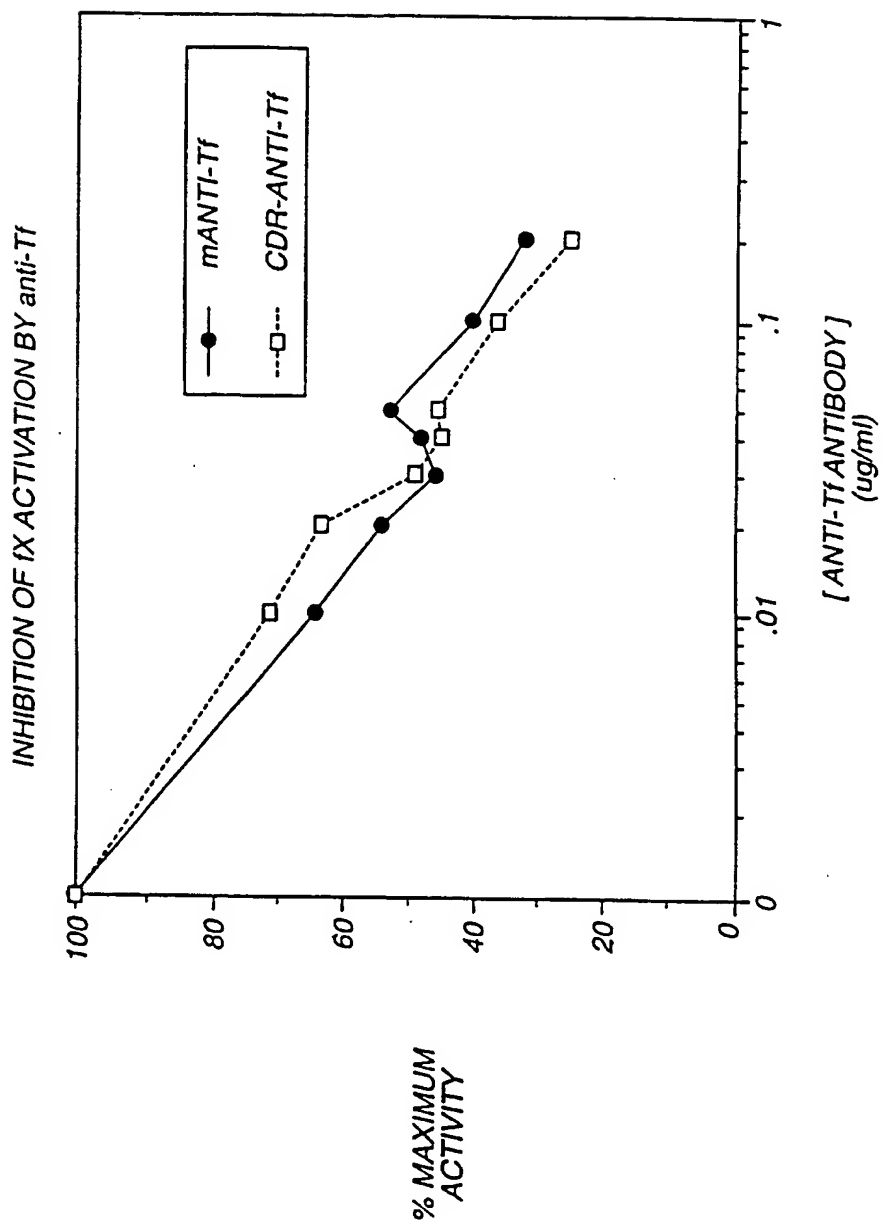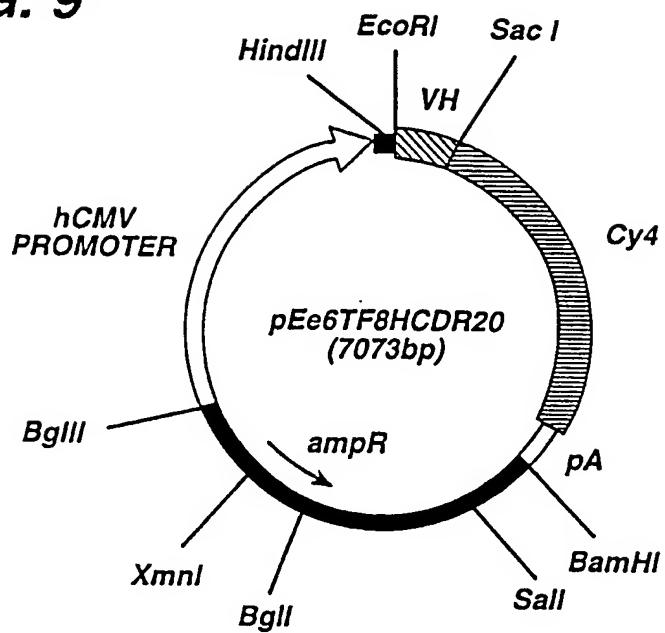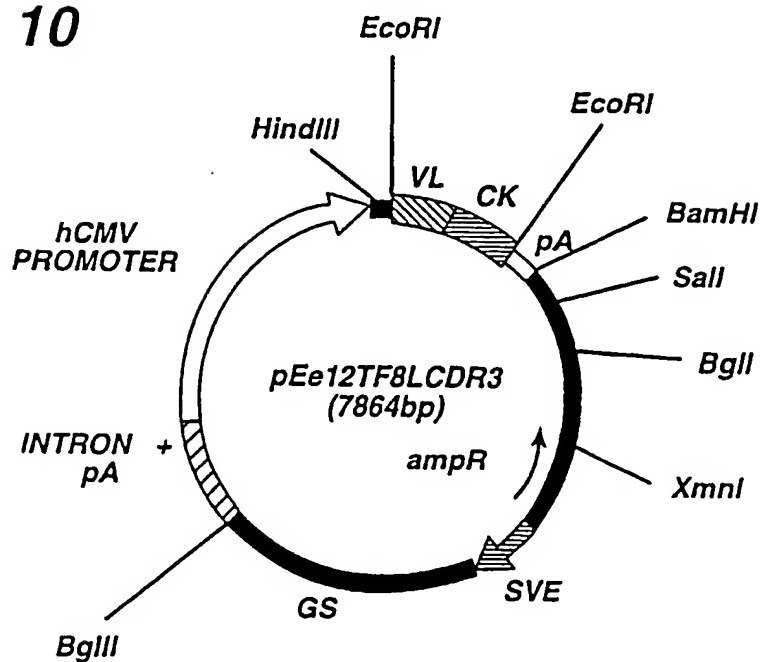
FIG. 6

anti-TF BINDING ASSAY

FIG. 7

anti-TF COMPETITION ASSAY

INHIBITION OF fX ACTIVATION BY anti-Tf

mANTI-Tf
CDR-ANTI-Tf

[ANTI-Tf ANTIBODY]
(ug/ml)

% MAXIMUM
ACTIVITY

FIG. 8

FIG. 9

FIG. 10

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C12N15/13   C07K16/36   C07K16/46   A61K39/395   //C12N5/10,
C12N15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C12N   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 91 09968 A (CELLTECH LIMITED) 11 July 1991 see examples see claims ---	1-37
Y	WO 88 07543 A (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 October 1988 see claims ---	1-37
A	WO 94 11029 A (THE SCRIPPS RESEARCH INSTITUTE ET AL.) 26 May 1994 see claims ---	1-37
A	WO 94 05328 A (THE SCRIPPS RESEARCH INSTITUTE) 17 March 1994 see examples see claims ---	1-37

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 October 1996	0 8. 11. 96

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Nooij, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF CRYSTAL GROWTH, vol. 122, no. 1-4, August 1992, AMSTERDAM, NL, pages 253-264, XP002015918 W. RUF ET AL.: "Purification, sequence and crystallization of an anti-tissue factor Fab and its use for the crystallization of tissue factor." see abstract see table 1 -----	1-37

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Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:   31-35
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 31-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

nformation on patent family members

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		AU-B-	649645	02-06-94
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